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Amines in Fish Muscle

III. Spoilage of Iced Eviscerated Cod

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(*Received for publication March 15, 1945*)

ABSTRACT

Bacterial counts, trimethylamine and trimethylamine oxide concentrations, and pH in gutted cod stored in melting ice show that spoilage originates in the belly cavity. Bacteria do not penetrate the muscle until spoilage is well advanced, but the substrates, trimethylamine oxide, etc., diffuse outward to the surface and the bacterial products diffuse rapidly into the muscle. Leaching by the ice water lowers the concentration of trimethylamine oxide at the skin. A gradient of high trimethylamine concentration in the belly cavity, decreasing through the muscle to low values at the skin, rapidly develops.

The fresh fish landed by the Atlantic schooner and trawler fishing fleet are gutted aboard ship, and stored in crushed ice soon after catching. These fish as landed were found on examination during 1944 to vary greatly from vessel to vessel in degree of preservation. Sampling of such fish necessitated a knowledge of the comparative spoilage rates of the several parts of the fish, a knowledge which is essential for researches on improvement of handling methods.

Hunter (1920) found that in salmon the bacterial count of the muscle of the belly wall was two to three times that of the back muscle. This same difference was also found by Harrison, Perry and Smith (1926) in mackerel, and by Birdseye (1929) in haddock. Harrison *et al.* also found that in haddock stored at 10° and 20°C. the bacteria in the subcutaneous back muscle increased slightly earlier near the gills than at the caudal end. Stewart (1931) found that the bacterial contamination of the blood in ungutted fish was low, and concluded that the skin is more important than the blood vessels as a muscle infectant. From the loss in volatile base in cod stored at 18°C. Beatty and Gibbons (1937) found that while the back muscle showed only a slight rise in one or two days, the peritoneum showed a considerable increase and the gills spoiled very fast. They attributed spoilage of iced fish to the bacteria working their way through the belly wall, and down the blood vessels, and in the case of haddock passing through the skin. Kiser and Beckwith (1944) also found that the bacterial count of the back muscle of mackerel was very much lower than that of the flank, and that the count of the heart blood was higher than that of the muscle, suggesting contamination through the blood system. This last was also found by Wood (1940).

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Careful work by various investigators (Harrison *et al.* 1926, Stewart 1931, Wood, Sigurdsson and Dyer 1942) has shown that the muscle regions farthest from the surfaces remain sterile up to the advanced stages of spoilage. The latter workers have shown that in stored fillets trimethylamine diffuses inward from the spoiling surface. Thus the diffusion of bacterial end products towards the sterile interior from the site of bacterial contamination on the surfaces of eviscerated fish stored in ice was indicated. The present investigation confirms this deduction.

METHODS

Sampling

Skin. Samples of the skin, about 5 by 7 cm. in area, were removed from the caudal section of the fish, from the nape or belly wall just posterior to the pectoral fin, and from the dorsal surface just beside the anterior portion of the median dorsal fin.

Muscle under skin. Samples of the muscle immediately under the skin, about 2-3 mm. in depth, were taken from the surfaces exposed by the removal of the three skin samples above.

Central muscle. Samples of the deep central muscle lying midway between the belly cavity and the skin surfaces were then taken at the same three positions.

Peritoneum. The peritoneum, black nape or lining of the belly cavity, was sampled.

Muscle along backbone. A sample of the muscle lying close to the backbone over the ribs at about the position of the kidneys was also taken.

Dorsal aorta. Samples of the dorsal aorta and the surrounding slime with a portion of the kidney tissue were taken near the forward end of the belly cavity.

Chemical

The excised samples, usually 5 or 10 g., were weighed and mixed with an equal weight of water and then with 10 per cent trichloracetic acid in amount twice that of the water. The mixture was triturated with a glass rod and shaken occasionally during a half hour. Trimethylamine was determined in an aliquot of the supernatant liquid by the colorimetric picrate method of Dyer (1945). The content of trimethylamine plus trimethylamine oxide was determined in another aliquot by Beatty's modification (1938) of the procedure of Lintzel (1934), the trimethylamine in the reduced solution being determined colorimetrically as above. For brevity, trimethylamine concentrations in mg. nitrogen per 100 g. tissue are termed the trimethylamine value.

Some of the trimethylamine values found were greater than the corresponding sums of trimethylamine plus trimethylamine oxide. It was found near the end of the investigation that the method for trimethylamine oxide was not satisfactory when only small amounts were present as in the later samplings. Satisfactory recovery of trimethylamine oxide in amounts of 2 to 40 mg. nitrogen could be obtained by making the solution up to a volume of 20 ml., adding first 1 g. Devarda's alloy and then 15 ml. 6N hydrochloric acid in 5 equal portions over a period of 50 minutes with the flask held in a water bath at 65°C. Re-

coveries varying from 50 to almost 100 per cent were obtained with amounts below 2 mg., the lower the concentration the lower the recovery. Consequently, while the trimethylamine oxide values obtained on the fresh fish samples are probably fairly accurate, the values may be quite low where the oxide is almost completely reduced or diffused out. Apparently a loss of preformed trimethylamine also occurs, since negative values of trimethylamine oxide are found in some of the results for 20 days. No satisfactory procedure has yet been devised for the determination of small amounts of trimethylamine oxide. Reduction by actively reducing strains of bacteria in peptone broth has been tried. Quantitative reduction to trimethylamine can be obtained in four to six hours, but the application of the method to fish tissue extracts has not yet been completely studied.

Surface pH was determined with the glass electrode, as described by Dyer, Sigurdsson and Wood (1944).

Bacteriological

Use of the strictest asceptic precautions and considerable practice in removing samples were found necessary to prevent contamination during sampling. After the removal of skin samples, the skin of one side of the fish was thoroughly scrubbed with dampened pads of cheesecloth to remove dirt, slime and scales. The skin was then swabbed with 0.5 per cent aqueous phenol, followed by 70 per cent alcohol, care being taken not to have an excess of either solution. Knives and forceps were sterilized before each cut by brushing with hot running water and soap, dipping in alcohol and flaming. Each sample was placed in 100 ml. sterile water in a weighed 250 ml. Erlenmeyer flask which was then reweighed. Dilutions were made with sterile tap water, and plates containing the equivalent of 2, 1, 0.1, 0.01, and 0.001 ml. of the original solutions were poured in duplicate at about 40°C. with a nutrient agar of the following composition:

Bacto-Peptone.....0.2%	Sodium chloride, c.p.....0.5%
Bacto-Yeast extract....0.2%	Dipotassium phosphate, c.p....0.1%
Bacto-Beef extract.....0.2%	Bacto-Agar.....1.5%
Bacto-Glucose.....0.1%	

Adjusted to pH 7.2 before autoclaving.

This medium was adopted because higher counts are obtained and the bacteria contaminating fish grew more luxuriantly than with standard nutrient agar. Approximately the same results are obtained with this medium as with the fish digest agar of Wood, Sigurdsson and Dyer (1942), and it is free from the small coagulated particles difficult to distinguish from small colonies which are present in the latter medium. The plates were counted after incubation at 20°C. for one and three days.

Experiment A

A preliminary experiment with iced fish showed large differences in the rate of trimethylamine formation in composite samples of the muscle of the flank, back, and tail. Therefore an experiment was set up to determine the relative spoilage rates in the various parts of the fish from the surface to the centre.

Six medium-sized codfish, approximately 30 inches (76 cm.) in length, landed by a schooner at Halifax, after having been stored several days in ice, were brought to the laboratory and packed in crushed ice. They were re-iced daily. Fish were removed after storage for 0, 3, 4, 5, 6, and 7 days, and sampled. Trimethylamine determinations only were made in this experiment.

The results obtained (figure 1) are striking. In the nape section, the peritoneum decomposed very rapidly as compared with the skin, in which no significant change in trimethylamine value was observed during the first three days. The trimethylamine value of the central muscle rose quite rapidly, whereas the muscle next the skin showed a later and smaller increase. Thus there is a steep

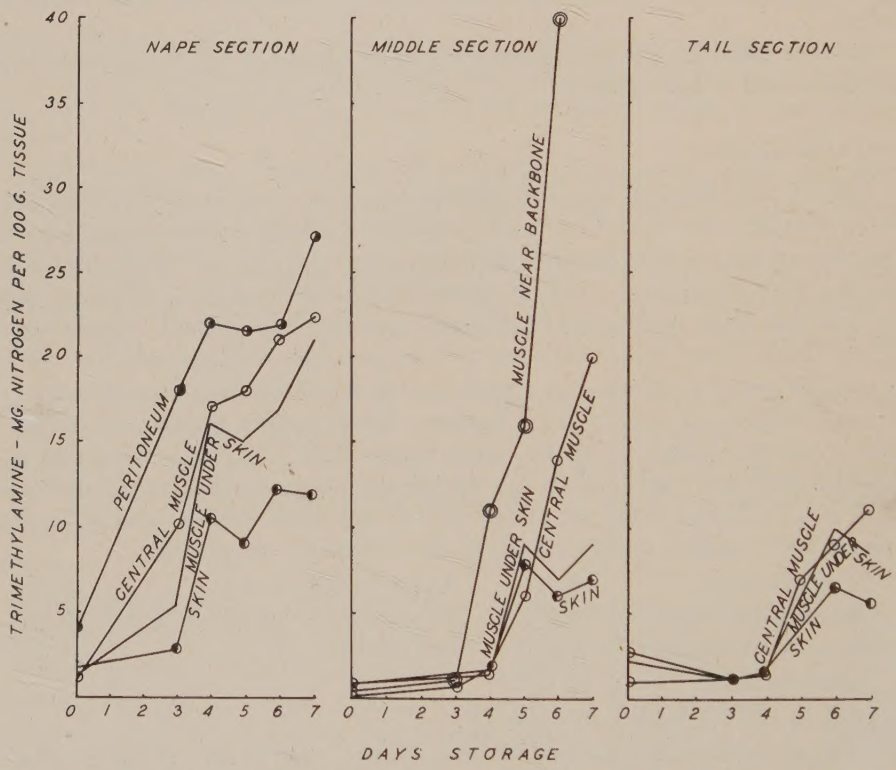


FIGURE 1. Experiment A. Trimethylamine rise in various tissues of cod stored in melting ice.

gradient from high values in the peritoneum to low values in the skin, suggesting that either the bacteria or their products, of which trimethylamine is a measure, enter the muscle through the rapidly decomposing peritoneum rather than through the skin. Thus the central muscle of the nape has reached the organoleptic threshold, which is usually at a trimethylamine level of 10 to 15, in 3 to 4 days, about two days earlier than the middle section.

In the middle section the spoilage rate was slower, no change having occurred up to four days except for the sample taken close along the backbone. Here, there was an extremely rapid increase in trimethylamine values from 1 to 11 between the third and fourth day. It had reached a trimethylamine value

of 54 at 7 days and was badly spoiled. The corresponding increase in the samples of the skin and the muscle under the skin was slightly lower than for the nape section. The trimethylamine increased in the central muscle at a rate median to the muscle along the backbone and that under the skin. Here again a diffusion of bacteria or spoilage products from the belly cavity through the spaces between the ribs and into the central muscle is indicated.

In the tail samples, the rise in trimethylamine values occurred later, but as with the previous sections the values of the centre and the muscle under the skin rose faster than those of the skin.

Thus all the evidence indicates that the principal source of infection and of spoilage is the lining of the visceral cavity. How does the spoilage of the interior muscle occur? Is the trimethylamine produced at the surfaces by reduction of trimethylamine oxide, which has diffused to the surface from the interior muscle, and does it then diffuse into the interior muscle? Or, do the bacteria gradually work their way into the muscle, particularly from the belly cavity?

To answer these questions an experiment was set up in which the concentration of trimethylamine and of trimethylamine oxide and the bacterial counts of the various portions of the fish were determined.

Experiment B

Trawler fish only one day on ice when received were immediately surrounded with crushed ice in a box covered with wet sacking and were kept very well iced throughout the duration of the experiment, samples being taken at intervals for bacteriological and chemical analyses. Samples for bacteriological analyses were removed from one side of the fish, and the other side was used for the samples for chemical analyses. Experiment A had shown that in well iced fish very little difference in the spoilage rates of the two sides of the fish occurred. Further, the smoothness of the curves obtained showed that with occasional exceptions, as are evident from the figures, only a small variation occurs in the spoilage rates of different fish.

The results of the examination of fish removed after 1, 10, 16, and 20 days are shown in table I. A preliminary experiment which was used to perfect the bacteriological sampling technique had shown that these four points are sufficient to chart the course of spoilage starting with fresh fish. The trimethylamine values (figure 2) confirm the observations made in experiment A showing the rapid spoilage of the lining of the belly cavity, the early trimethylamine rise in the nape and along the backbone, and the progressively lower values as the skin is approached from the interior. In the tail section spoilage develops later, and in this experiment the trimethylamine values are twice as high in the centre as at the surface, indicating that the trimethylamine is produced in the centre and diffuses outwards. The very rapid spoilage in the region of the dorsal aorta is also well illustrated. Thus the same general picture of spoilage has been obtained in three separate experiments using 20 or more fish.

Spoilage of the Nape

The nape muscle remains practically sterile up to about 16 days, when a low count is obtained, whereas the trimethylamine values have reached their

TABLE I. Trimethylamine (TMA) as mg. nitrogen per 100 g., trimethylamine oxide (TMAO) as mg. nitrogen per 100g., bacteria (Bact.) as thousands per g. and pH in gutted cod stored in melting ice.

	July 12—1 day			July 21—10 days			July 27—16 days			July 31 20 days	
	TMA	TMAO	Bact.	TMA	TMAO	Bact.	TMA	TMAO	Bact.	TMA	TMAO
Nape											
Skin	0.87	31.	322	5.6	9.5	18,500	8.6	(−1.8)	2,000	9.2	(−5.6)
Under skin	0.45	66.	0	6.0	22.	0	14.5	(−2.0)	3	12.3	(−5.2)
Centre	0.35	76.	0	11.0	25.	0	20.	0	30	12.3	(−5.2)
Peritoneum	1.89		1100	25.		10,000	28.		100,000	18.	
Tail											
Skin	0.66	18.	140	0.82	11.	5,500	7.4	3.6	9,000	11.3	(−5.2)
Under skin	0.73	56.	0	1.1	32.	0	13.1	9.	2	15.	0
Centre	0.46	81.	0	1.1	65.	0	20.4	14.	0.9	20.	0
Middle											
Skin	0.70	21.	19	1.9	17.	24,000	9.6	0	450	18.	(−8.)
Under skin	0.43	71.	0	2.1	36.	0	14.	10.	32	24.	0
Centre	0.42	73.	0	1.3	66.	0	22.	5.	0	34.	(−2)
Backbone—											
forward	0.20	85.	0	5.2	61.	0	36.	2.	1	37.	0
kidney	0.32	85.	0	8.1	56.	0	39.	0.	220	39.	(−7)
Dorsal aorta	4.0		0*	31.		1.7	33.		200		
		pH			pH			pH			pH
Nape—centre		6.5			7.0			7.0 −7.2			7 − 7.3
Middle—centre		6.5			6.7			7.0			7 − 7.3
Middle—skin		7.0			7.2			7.2			7 − 7.3

*Dorsal aorta at 6 days: Trimethylamine, 22; bacteria, 8000 per g.

maximum at this time. The skin and the peritoneum are very highly contaminated, and these data show that very little migration of the bacteria occurs either through the thin peritoneum or through the skin. Instead the products of bacterial action diffuse through the muscle. Analyses show that about two-thirds of the trimethylamine oxide has diffused from the central muscle in 10 days and all has been removed in 16 days. Thus 76 mg. of trimethylamine oxide nitrogen per 100 g. has diffused from the interior to the surface and 20 mg. of trimethylamine nitrogen per 100 g. have diffused back to the centre. Accordingly about 50 mg. nitrogen per 100 g. in the form of either trimethylamine or trimethylamine oxide, or both, has been leached from the centre of the nape by the water from the melting ice. Between 16 and 20 days an additional 8 mg. trimethylamine nitrogen per 100 g. has diffused from the interior into the leaching water and the trimethylamine content of all the muscle layers has thus decreased, the skin remaining about the same.

SPOILAGE OF THE MIDDLE

The tissues in the region of the dorsal aorta spoil very rapidly, even earlier than and as fast as the peritoneum. At one day 4 mg. trimethylamine nitrogen per 100 g. muscle has been produced and this has increased to 31 and 33 at 10 and 16 days. This forms a supply for diffusion of bacterial products to the back muscle through the spaces between the ribs. The trimethylamine values in this case were determined by distillation, (Beatty and Gibbons 1937, Dyer and

Mounsey 1945), since at 16 days considerable amounts of nonvolatile amines were produced, about the equivalent of an amine value of 30 expressed as trimethylamine. The bacterial counts of the dorsal aorta are not as high as expected, probably because the membrane between the dorsal aorta and belly cavity impedes early contamination. In the later samples the dorsal aorta had been decomposed and high counts were obtained. Also the results of analysis of the back muscle along the backbone indicate that the site of maximum bacterial action is probably in the region of the kidneys near the caudal end of the belly cavity. A rapid rise in the trimethylamine content of the muscle adjoining the backbone occurs in 10 to 16 days. Bacterial invasion of the muscle over the kidney region has occurred at 16 days while it is just starting in the muscle over the forward part of the belly cavity. Organoleptic observations show that the

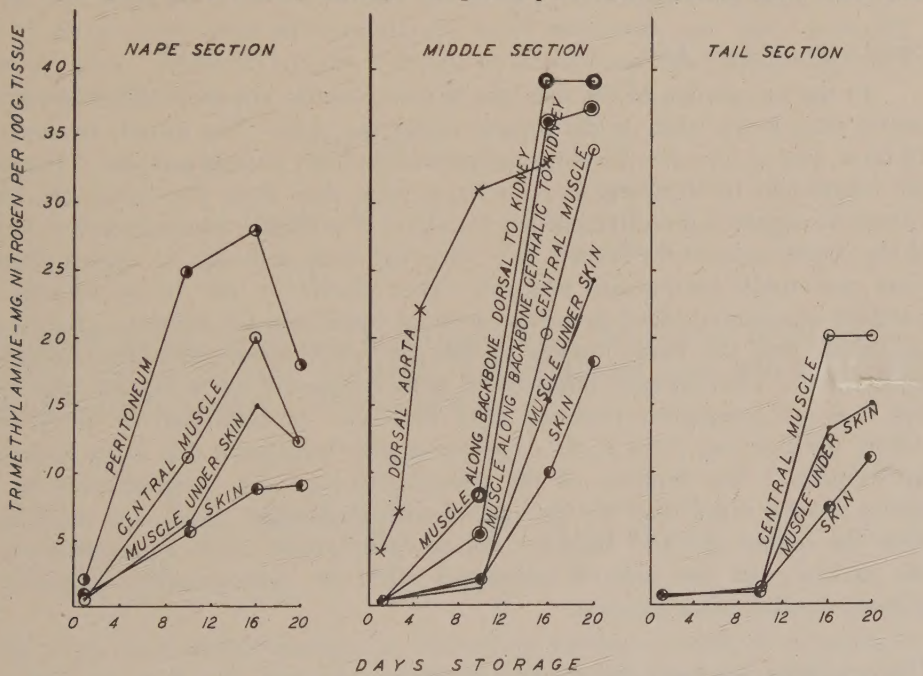


FIGURE 2. Experiment B. Trimethylamine rise in various tissues of cod stored in melting ice.

kidney region decomposes very rapidly, and it is in this region that the red streaks in the flesh observed in spoiled fish first appear.

Following the rapid rise of the trimethylamine in the muscle along the backbone between 10 and 16 days, diffusion of the amine into the central muscle quickly occurs as shown in the curves and a gradual rise in trimethylamine concentration occurs in the muscle under the skin and in the skin. These data show that the bacteria on the skin slime play a very minor role indeed in contributing to the trimethylamine of the muscle, since the trimethylamine could not diffuse into the central muscle against the concentration gradient. Undoubtedly heavy bacterial growth does occur in the slime of the skin, but the trimethylamine produced is probably washed off by the ice water and probably some leaching of the trimethylamine diffusing out from the centre also occurs.

The skin provides an effective barrier to the entrance of bacteria, the muscle under the skin remaining sterile up to 10 days, but showing some contamination at 16 days with the centre still sterile. No bacterial counts were made at 20 days.

The trimethylamine oxide data show the diffusion of this compound from the centre, about 30 per cent being leached out at 10 days. It has been almost completely removed at 16 days. At the same time trimethylamine has diffused back in to reach a trimethylamine value of about 20. Thus by the time bacteria have gained entrance to the area beneath the skin, almost all of the trimethylamine oxide has disappeared. It is interesting to note that Shewan (1939) also found a decrease in the sum of trimethylamine and trimethylamine oxide in haddock stored in melting ice, and this was assumed to be at least partly due to solution in the ice water.

Spoilage of the Tail

In the tail section of the fish, the trimethylamine content rises faster in the centre than in the skin or the muscle under the skin. The muscle is sterile at 10 days, but at 16 a few bacteria are present in both muscle samples. The data are insufficient to show whether the bacteria worked their way down the blood vessels or migrated directly through the skin. The higher trimethylamine values of the centre support the former view, although some diffusion of trimethylamine from the middle section has probably taken place. It may be noted that the partially coagulated blood provides excellent conditions for bacterial growth and migration into the blood vessels at the tail, with subsequent trimethylamine production. The outward diffusion of trimethylamine oxide occurs here also, but it is not completely removed until 20 days. At least part of its decrease during the last four days of the experiment may have been due to its reduction by bacteria. The decrease of the trimethylamine oxide concentration in the centre is more rapid than the increase of trimethylamine. This is in accordance with the higher gradient between the trimethylamine oxide concentrations at the surface and the interior compared with the corresponding gradient in trimethylamine concentration.

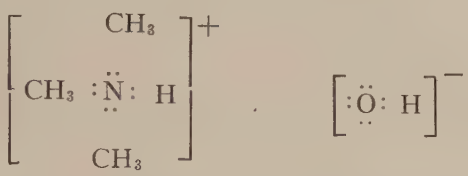
Comparative Spoilage Rates in the Nape, Middle, and Tail

The rapid rise in trimethylamine occurred at 6 to 10 days in the nape, 10 to 12 days in the main body of the fish, and at about 12 to 14 days in the tail. Thus the nape was spoiled 3 to 4 days before the thick part of the flesh and 5 to 6 days before the tail muscle. The results of the pH determinations also showed the same relation, the pH of the interior nape muscle rising to 7 at 10 days whereas the middle muscle has increased only from pH 6.5 to 6.7. This difference then decreased as spoilage progressed, probably owing to the relatively greater effect of diffusion and washing out in the nape. Under commercial conditions, the quality of the nape should thus be a guide to the quality of the main body of the flesh, a rise in the amine content of the nape indicating that the fillet will also be spoiled in another 2 to 4 days depending on the relation which may be found applicable to actual operating conditions. Most of the thin nape goes into scrap and is not utilized in cutting fillets, and the mixture of the spoiled

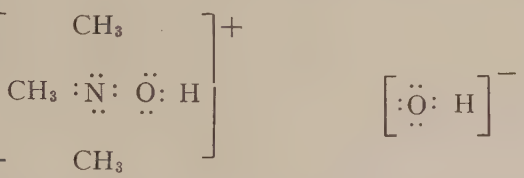
nape with the remainder of the sound flesh is thus happily avoided in practice. As regards sampling methods, the nape obviously does not give a true picture of the quality of the fillet. Probably the best sample is obtained by cutting across some part of the back muscle, since the tail is apt to be of slightly better quality.

DISCUSSION

These experiments have shown that trimethylamine oxide diffuses rapidly throughout the tissues. As it is reduced to trimethylamine by the bacteria at the exposed surfaces, the skin and the visceral cavity, diffusion to these surfaces results in a rapid lowering of the oxide concentration in the interior tissue. Simultaneously a diffusion of trimethylamine to the central muscle occurs. Also the fish loses both the amine and the oxide by diffusion into the ice water. Both of these substances have relatively small molecules and they probably diffuse as ions. Trimethylamine, a much stronger base than ammonia, adds water to form the ions



The hydroxyl ion will be replaced by lactate or some other cation in the neutral or slightly acid fish muscle. Similarly, trimethylamine oxide also ionizes in neutral solution by adding a molecule of water



As with the trimethylamine, an equivalent amount of acid is required to neutralize the hydroxyl ion and replace it with lactate or some other cation before an acid solution can be obtained. This accounts for the relatively high buffering power of trimethylamine oxide in fish muscle, which was found by Cutting (1939) and Collins, Kuchel and Beatty (1941). The decrease in buffering power of stored fish used by Stansby and Lemon (1933) as a freshness test is due mainly to the lowered concentration of trimethylamine oxide which we have shown to occur both by reduction to the amine and by diffusion out of the muscle. Thus a rise in pH would be expected in the interior tissue with the removal of the oxide buffer and increase in the basic trimethylamine, particularly since, as has been shown to occur in the later stages of the spoilage of stored cod fillets (Wood Sigurdsson and Dyer 1942), lactate also probably diffuses out along with the oxide. Since the outward diffusion of trimethylamine oxide and probably lactate occurs quite early, and the trimethylamine rapidly increases in the nape tissues, there is an appreciable early rise of pH which might perhaps be sufficient

to serve as a rapid quality test for use on gutted fish as landed. The relation of spoilage times of the nape and the main thick muscle would have to be determined under varying commercial conditions before its usefulness could be ascertained.

Thus spoilage in gutted cod and similar fish originates principally in the belly cavity. The first tissues to show signs of decomposition are the gills. These are followed closely by the peritoneum, the surface slime, and the tissues ventral to the backbone—the kidneys and the dorsal aorta. Very rapid bacterial growth occurs in these regions, and the products of their action rather than the bacteria themselves diffuse into the flesh in the napes and pass between the ribs into the body muscle. Simultaneously a movement to the surface of trimethylamine oxide and probably lactic acid, etc. occurs, to replace that utilized by the bacteria. Most of the trimethylamine produced in the surface of the skin is washed off by the ice water while that produced in the surface of the visceral cavity diffuses into the muscle. The bacterial counts show that the skin is a good barrier to infection, although it is quite permeable to the diffusion of ions or salts, such as those from trimethylamine or trimethylamine oxide. The skin of cod or haddock has been shown to offer no more resistance than the flesh to the movement of sodium chloride or water (Dyer 1942).

Another consequence of the sterility of the muscle tissue is that the interior of the fillets when cut will be sterile, but the surface will be contaminated with the bacteria from the fish, cutting table, knives, boxes, conveyors, etc. Subsequent spoilage will thus be from the surface bacterial growth, and the trimethylamine produced will augment that which was already present before cutting.

The earliest bacterial penetration of the flesh occurs near the kidney region, and it is here that the first signs of the red streak often observed in spoiling fish starts, spreading both forward and backward along the backbone from the point of entry. Obviously, therefore, thorough washing of the belly cavity at the time of gutting, with complete removal of the viscera, would result in less initial contamination with consequent improved keeping quality.

Considerably more ice was probably used in the above experiments than that used aboard the fishing vessels, and thus a greater amount of leaching out of trimethylamine oxide has probably occurred than that obtaining in practice, but the conclusions reached above will still apply. However, where considerable leaching does occur, as it undoubtedly does in certain parts of the storage pens, the oxide content of fillets cut from older fish may be quite low so that the trimethylamine development on the spoilage of the fillet may be lower than usual.

The fish iced in the laboratory, experiment B, kept for about 12 days before reaching a trimethylamine value of 10 mg. nitrogen per 100 g. muscle. The analyses of over 5,000 samples from 205 landings of trawlers and vessels during 1944 (unpublished results) showed that on the average the fish took only six to eight days to reach the above level. The use of more ice in the laboratory experiment kept the fish temperature at 32°F. (0°C.) as compared with temperatures of 32°F. to 44°F. (0°C. to 6.7°C.) with an average temperature of about 34°F. (1.1°C.) observed on the vessel fish. Hess (1932) has shown that near the freezing point, a small decrease in temperature is very effective in reducing the activity of bacteria in fish, and therefore the difference between 34°F. and

32°F. is very significant. Obviously methods of icing and washing are important factors in spoilage.

SUMMARY

The mode of spoilage of gutted codfish stored in crushed ice has been followed by determination of bacterial count, trimethylamine, trimethylamine oxide and pH on samples of various parts of the fish.

The nape was found to spoil earlier than the middle muscle, and the tail section was the last to show decomposition.

Most of the spoilage originates by bacterial decomposition of the tissues in the lining of the belly cavity, rather than from bacterial growth on the skin. Bacteria do not penetrate the skin until very late in the spoilage process, and the migration through the lining of the belly cavity occurs at about the same time, about 16 days in the experiment reported.

The significant conclusion is that the products of bacterial action and not the bacteria diffuse into the muscle, the central muscle remaining sterile for a considerable period even though the trimethylamine may have almost reached its maximum value.

The peritoneum and the dorsal aorta with the tissues adjoining the latter decompose early and very rapidly. A gradation of trimethylamine concentration, from high values in the tissues of the belly cavity through the intermediate values in the central muscle to low values in the muscle under the skin. and the skin, rapidly develops in all sections of the fish. Water from the melting ice lowers the trimethylamine concentration in the skin. Trimethylamine oxide diffuses outward from the centre to replace that reduced to trimethylamine by bacteria or washed out at the surfaces, gradually reducing the oxide concentration to approximately zero at 16 to 20 days.

Limitations to the Lintzel method of trimethylamine oxide determination are noted.

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Colorimetric Nitrite Determination

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ABSTRACT

Sodium nitrite is quantitatively extracted from fish tissue by water in the Waring blendor. An accurate colorimetric method for nitrite determination has been developed using sulphanilamide and N-(1-napthyl) ethylenediamine dihydrochloride. The red colour is stable from five minutes to several hours.

Many modifications of the Griess test (1879) have been used in which nitrite has been diazotized with sulphanilic acid or a similar compound, with subsequent coupling with a naphthylamine derivative resulting in the formation of a highly coloured azo dye. The introduction of more satisfactory reagents has been stimulated by the slow development of colour, rapid fading, and precipitation of the coloured complex characteristic of the original procedures. Bratton and Marshall (1939) investigated various coupling reagents, and introduced the use of N-(1-naphthyl)-ethylenediamine dihydrochloride for the determination of sulphanilamide. The azo compound produced was stable and more soluble, and the time of coupling was reduced to about two minutes. Shinn (1941), and Kershaw and Chamberlin (1942) used this compound for the determination of nitrite in water samples. The final colour is a measure of either the nitrite or the diazotization agent, whichever is present in limiting concentration.

Rose and Bevan (1944) used N-β-sulphato-ethyl-m-toluidine as a coupling reagent for sulphanilamide estimation but its use has not been extended to nitrite analysis.

White (1939) used a photoelectric colorimeter with a filter, maximum light transmission at 5200 A.U., his reagents being more concentrated than used in the standard A.O.A.C. method (1940, p. 527). Looney and Dyer (1942) also used a 5200 A.U. filter with sulphanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride reagents in 30 per cent acetic acid. The methods of Shinn, and of Kershaw and Chamberlin have been studied by the use of the photoelectric colorimeter, and simple, accurate procedures developed.

Reagents

A. sulphanilamide

0.2 g. p-aminobenzene sulphonylamide dissolved in 20 ml. concentrated hydrochloric acid and 80 ml. water.

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B. COUPLING REAGENT

0.02 g. N-(1-naphthyl) ethylenediamine dihydrochloride dissolved in 100 ml. water. Store in a dark bottle, preferably in the refrigerator.

PROCEDURE

For 10 ml. final volume as used in the photoelectric colorimeter, dilute 0.5 ml. tissue extract (see below) or an aliquot of sample solution containing 0.0002 to 0.005 mg. sodium nitrite (0.00005-0.001 mg. nitrite nitrogen) to 10.0 ml. in a colorimeter tube. The sample solution should be neutral or slightly acid. Add 1 ml. sulphanilamide reagent and mix thoroughly. Follow by 1 ml. coupling reagent and again mix thoroughly. The red colour is measured in a photoelectric colorimeter using a filter with maximum transmission at 5400 A.U. A 5200 filter may be used satisfactorily but the absorption is not then at a maximum (figure 1).

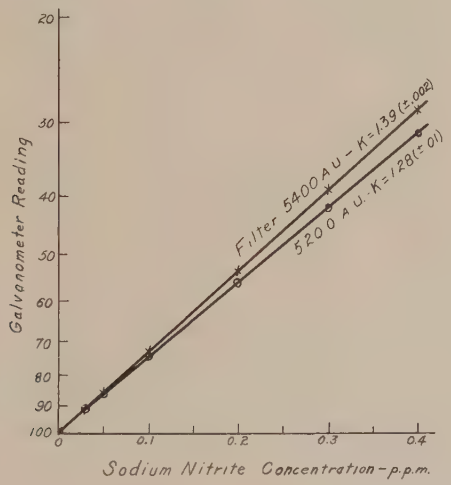

FIGURE 1. Standard curve for nitrite-sulphanilamide-N-(1-naphthyl)-ethylenediamine dihydrochloride colour (per cent light transmission, logarithmic scale, plotted against sodium nitrite concentration in p.p.m.).

Brattan and Marshall also found the absorption maximum of the red complex at 5450 A.U. Maximum colour development takes place in five minutes and the colour is stable for two to three hours with very little fading during 24 hours.

For reading, the colorimeter is set at 100 with a blank in which 1 ml. of water has been substituted for the sulphanilamide reagent. For standardization of the colorimeter, a sodium nitrite solution is prepared from silver nitrite (A.O.A.C. 1940, p. 527) and a standard curve prepared by using various dilutions (figure 1). More conveniently the constant K may be computed. K was found to be 1.39 ($\pm$0.002) where K equals 2 - log galvanometer reading divided by the concentration of nitrite expressed as parts per million (p.p.m.) sodium nitrite in the 10 ml. volume. Beer's law is applicable over the range given. If only a few determinations are to be made, the colour given by 10 ml. of a standard solution con-

taining 0.20 p.p.m. sodium nitrite is measured, and the concentration in the unknown sample is then

$$\frac{2 - \log G \text{ of sample}}{2 - \log G \text{ of standard}} \times 0.20 \text{ p.p.m. sodium nitrite}$$

If the extraction method given below is used (10 g. fish diluted to 500 ml.) and 0.5 ml. extract is used per tube, the factor 200 instead of 0.20 should be used in the above equation.

This method is now in use in the laboratories of several fish processing plants.

Use of Nessler Tubes

For the use of 50 ml. Nessler tubes, as in water analysis, the sample should be diluted to 50 ml., followed by the addition of 2 ml. of 0.5 per cent sulphanilamide in 1:1 hydrochloric acid and 1 ml. of 0.1 per cent coupling reagent as before. These reagents offer considerable advantage over the Griess reagents in that the colour may be compared at any time from five minutes to several hours.

Spot Test

The test may be used with a drop of solution on a spot plate, sensitivity about 0.1 p.p.m., or directly on the tissue surface, or the surface of a solution in a beaker, by adding a drop of each reagent.

Extraction of Nitrites

White (1939) obtained results within one per cent of those obtained using the A.O.A.C. method (1940, p. 378) by freezing and thawing the minced tissue and shaking a 10 g. sample for two hours with about 150 ml. of water at a temperature of about 80°C. These methods are long and cumbersome for routine work, and the following simplified methods were tried on samples of well mixed minced fillets of cod and haddock:

A. A.O.A.C. method using 10 g. fish.

B. 10 g. fish plus water to 500 ml. volume and blended two minutes in the Waring blendor.

C. 20 g. fish plus 300 ml. water, blended as above, and finally diluted to 400 ml. volume.

D. 10 g. fish plus water to 500 ml. volume and shaken occasionally during about one half hour.

E. 10 g. fish added to 50 ml. 0.2 N acetic acid-sodium acetate buffer, pH 4.5, and diluted to 500 ml. with water, and blended.

F. Same as E except the mixture was shaken occasionally during a half hour instead of blending.

G. 50 g. fish plus water to 500 ml. volume and blended as above.

H. 10 g. fish added to 5 ml. formalin solution, the mixture diluted to 500 ml. with water, and blended as above.

Extractions were made in triplicate with duplicate nitrite analyses on each extract.

The results (table I) show that all methods of extraction gave comparable results except method C. In this case the froth produced during blending prevented the solution from being made to volume accurately, and low results were obtained. Since blending the sample, as in method B, is so much simpler than the A.O.A.C. method, it has been adopted for work on fish muscle and has proven satisfactory over a period of three years. The fine degree of dispersion obtained by the use of the blendor would probably render the method applicable to meats

TABLE I. Comparison of extraction methods, as to the nitrite found.

Method	In p.p.m. of fish				%, based upon A = 100% Av.
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	
	Haddock	Cod	Cod	Cod	
A. A.O.A.C.	94 (± 2)	229 (± 7)	264 (± 6)	269 (± 4)	100%
B. 10 g. in 500 ml., blended	94 (± 2)	227 (± 6)	268 (± 9)	260 (± 3)	99
C. 20 g. in 300 ml., blended, to 400 ml.		201 (± 4)	244 (± 5)		90
D. 10 g. in 500 ml., shaken	95 (± 1)	228 (± 5)	268 (± 7)	265 (± 3)	100
E. 10 g. + buffer in 500 ml., blended	96 (± 2)	224 (± 2)	255 (± 2)		99
F. 10 g. + buffer in 500 ml., shaken	98 (± 3)	226 (± 4)	250 (± 9)		99
G. 50 g. in 500 ml., blended				262 (± 3)	98
H. 10 g. + HCHO in 500 ml., blended				263 (± 2)	98

as well as fish. Analysis of the extract should be carried out without delay since bacterial action results in an appreciable reduction of the nitrite content even on standing overnight at room temperature (10-50%). The inclusion of the formaldehyde, method H, prevents bacterial growth, and it may be added if necessary.

The recovery of known amounts of sodium nitrite added to samples of cod fillets is shown in table II. The equivalent of 250 p.p.m. sodium nitrite was added to weighed portions of fillet and extractions on fillets with and without nitrite addition were made as follows:

A. 10 g. fish extracted by the A.O.A.C. procedure.

B. 10 g. fish diluted to 500 ml. with water and blended.

C. 20 g. fish made up to 500 ml. with water and blended.

Quantitative recovery of added nitrite was obtained, 98 to 101 per cent, with all procedures.

TABLE II. Recovery of added nitrite.

Method	$NaNO_2$ found	$NaNO_2$ added	$NaNO_2$ recovered	
	(p.p.m. of fish)	(p.p.m. of fish)	(p.p.m. of fish)	(per cent)
A. 10 g. fish A.O.A.C.	170 (± 4)			
10 g. fish A.O.A.C. + $NaNO_2$	422 (± 7)	250	252	101
B. 10 g. fish—500 ml., blended	170 (± 5)			
10 g. fish—500 ml., blended + $NaNO_2$	420 (± 7)	250	250	100
C. 20 g. fish—500 ml., blended	164 (± 4)			
20 g. fish—500 ml., blended + $NaNO_2$	409 (± 8)	250	245	98

SUMMARY

A simple colorimetric method for nitrite determination using sulphanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride as diazotizing and coupling agents has been developed. The red colour develops within five minutes and is stable for several hours. Extraction of fish samples with water in the Waring blendor gave complete extraction, and added nitrite was recovered quantitatively.

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On *Themisto libellula* in Baffin Island Coastal Waters

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(*Received for publication March 28, 1945*)

ABSTRACT

The gross internal anatomy, food habits, local summer ecology and breeding cycle of *Themisto libellula* Mandt are studied in material from Baffin island coastal waters obtained in 1939 and 1940. The chitinous proventriculus projects backwards into the cavity of the midgut and forms a "gastric apparatus" which cuts off part of the wall of the anterior midgut from direct contact with the food. The gastric apparatus is strongly reminiscent of the peritrophic membrane in insects and the Onychophora. The food in the summer consists mainly of the smaller crustacean plankton; plant matter was also found in the gut. There is a high incidence of infection by a Gregarine parasite. The possible causes of the reduction in numbers of *Themisto* in the fjord waters in August are discussed; the reduction is tentatively referred to the agency of the outflowing surface current coupled with the habit of *Themisto* of seeking the surface waters. The breeding cycle is of the alternating, or two-phase type which has been recorded by the writer in other arctic planktonic forms. There is a breeding period in the early part of the year and perhaps another in the autumn.

INTRODUCTION AND MATERIAL

The study of the marine plankton has reached a stage at which the zoogeographical and systematic foundation laid in earlier years is being overlaid in patchwork fashion by more detailed investigation of the interrelations of the organisms concerned and of the biology of the individual members within the planktonic sphere. It is only by this means that a true picture of the complexity of the pattern of life in the sea, and hence the formulation of laws governing that life, can be achieved. It is natural and proper that the temperate zones have received the most attention, but the special properties of the arctic and antarctic environment endow this line of research with special interest, and since much can be learned from a comparison of the same or similar organisms in different natural conditions, it is essential that the polar work should not be allowed to fall too far behind the temperate.

The material for the present study was collected during the summers of 1939 and 1940, when the writer accompanied the Canadian Government Eastern Arctic Patrol as marine biologist. The bulk of the *Themisto* material was obtained at Lake Harbour, in southern Baffin island, during part of July and August of both years, and at Frobisher Bay in August 1940. The Lake Harbour collecting stations, with which the present paper is most concerned, are shown in figure 1. Smaller collections from River Clyde, Arctic Bay, Pond Inlet and Pangnirtung were made in both seasons. The total Baffin island material consists of 6,872

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specimens, of which 6,254 were taken at Lake Harbour and 538 at Frobisher Bay. Additional material was obtained from stomachs of the ringed seal *Phoca hispida*, at Lake Harbour, Frobisher Bay and River Clyde. With the exception of a quite inconsiderable quantity taken in silk nets, and of the large seal stomach material, the whole collection was made with the stramin one-metre ring-trawl, by horizontal hauls from a small motor-boat, at depths from the surface to approximately 40 metres. Below this depth plankton of all kinds was found to be scarce.

For hydrographic information on the region investigated the reader is re-

FIGURE 1. Lake Harbour stations (After J. D. Soper: Lake Harbour district, Southeastern Baffin island. Department of the Interior, Canada; Lands, Northwest Territories and Yukon Branch, 1931).

ferred to an earlier paper (Dunbar 1942). Temperatures and salinities taken at Lake Harbour, Frobisher Bay, River Clyde and Pangnirtung show that the water is typically arctic and coastal. At depths between 10 and 75 metres the temperatures during July, August and September ranged from −1.39 to +1.1°C. The highest surface temperature recorded was 3.68°C. at Lake Harbour on July 31, 1940. Salinities ranged from 32.93 to 27.83‰ in the fjord waters; a sample from Hudson strait, depth 75 metres, had a salinity of 33.62‰.

There is no doubt that in the eastern arctic of Canada *Themisto libellula* is

one of the most important organisms in any habitat, terrestrial or aquatic. Its ecological position might in many respects be compared with that of the lemming, and to the native population it is of even greater significance than the lemming, for it figures as the most common item in the food of the ringed seal, at least in the summer months (Dunbar 1941a). It is comparable also to the key planktonic species of the antarctic, *Euphausia superba* Dana, and to some extent seems to supplant the Atlantic"krill" (various euphausiids, including *Thysanoessa inermis*, *T. raschii*, and *Meganyctiphanes norvegica*) in arctic water.

OBSERVATIONS OF THE INTERNAL ANATOMY

Specimens of *Themisto* were dissected (fig. 2) for gut contents and gonads. The internal anatomy of the genus has never been described, and since the present paper is in fact the first in which *Themisto* has come up for special and detailed attention, a short description of the gross internal anatomy, including points of interest in the structure of the alimentary canal, is inserted here. The foregut is chitinous and consists of a short muscular pharynx and a proventriculus (fig. 2);

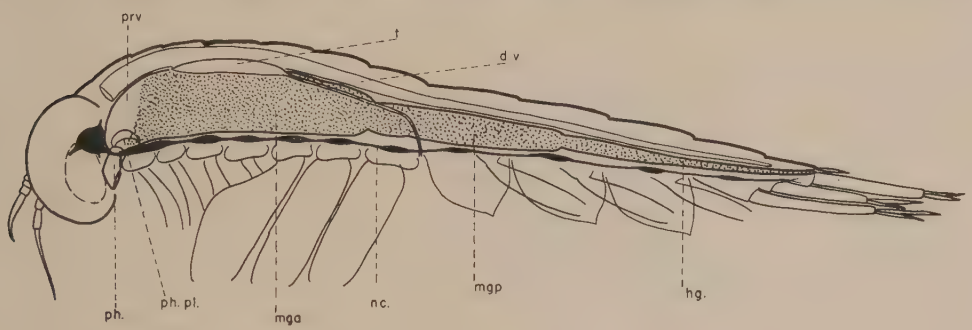

FIGURE 2. Diagrammatic dissection of *Themisto libellula*. The circumintestinal fat has been removed. d.v., dorsal blood vessel; hg, hindgut; mga, mgp, anterior and posterior midgut; nc, nerve cord; ph, pharynx; ph. pl., pharyngeal plates; prv, proventriculus; t, testis.

the midgut is long and divided into anterior and posterior portions, the constriction between the two portions occurring in the seventh thoracic segment. The chitinous hindgut extends from the second abdominal segment to the anus beneath the telson. The whole of the proventriculus and the midgut is surrounded by a heavy reserve of fat. The opening from the pharynx to the proventriculus is guarded by two pairs of toothed plates, as in *Gammarus* (Cussans 1904). There are no caeca or diverticula of any kind.

To give greater detail (fig. 3): The proventriculus is thin-walled and transparent; it is composed of two layers, an outer chitinous layer stained brown with gold chloride and silver nitrate (J. Holtfreter, unpublished), and an inner cuticle largely unaffected by the gold and silver technique. The midgut is attached to the proventriculus in the region of the first thoracic segment, so that the proventriculus continues backwards inside the midgut. This posterior portion of the proventriculus, within the midgut and lying free in the midgut cavity, or stomach, takes the form of a dorso-lateral sheet tapering posteriorly and dividing into two elongated processes. There is a ventral sheet extending a little

beyond the level at which the dorso-lateral sheet divides. The whole structure within the midgut, the posterior proventriculus, has been named for convenience the gastric apparatus (*ga*, fig. 3).

A structure similar in general to the gastric apparatus of *Themisto* but differing considerably in detail, has been described in the Phronomidae by Claus (1875). The apparatus appears to be restricted, in the Crustacea, to the hyperiid Amphipoda, and no comparable organization has been found in the better known Gammaridea. This is the more interesting in that it closely resembles, in principle, the peritrophic membrane in the Insecta and the Onychophora, a tubular chitinous membrane which is proliferated from the posterior end of the foregut and which projects backward as a lining in the midgut.

A portion of the wall of the anterior midgut is thus cut off from actual gross contact with the food in the gastric cavity, by the dorso-lateral and ventral plates of the gastric apparatus. In the pocket formed between the gastric apparatus and the midgut wall, and on the inner side of the midgut wall, are large droplets of fatty substance consisting of a mixture of phospholipids and unsaturated fats,

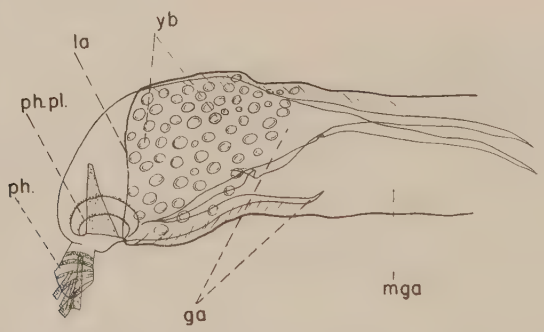

FIGURE 3. Anterior end of alimentary canal. ga, gastric apparatus; la, line of attachment of anterior midgut to proventriculus; ph, pharynx; ph. pl., pharyngeal plates; mga, anterior midgut; yb, "yellow bodies". Muscles of pharyngeal region stippled.

with a high proportion of the former. The droplets are sometimes yellow and opaque, sometimes clear and transparent, but usually of the same consistency in any one individual. The less opaque droplets contain varying quantities of liquid, possibly digestive enzyme, which is not affected by the fat or lipoid stains. It is probable that these fatty droplets, or "yellow bodies," are digestive in function, and that the gastric apparatus serves to facilitate the flow of digestive juices in the stomach. The whole system is of considerable intrinsic interest, and will be dealt with in detail in a later paper.

Figure 2 shows the dorsal blood vessel and the ventral nerve cord. The large suboesophageal ganglion represents the fusion of several ganglia including those serving the first two thoracic segments. The gonads lie dorso-lateral to the gut, the oviducts evacuating to the exterior by paired openings on the fifth thoracic segment, the sperm ducts on paired papillae close to the mid-ventral line on the seventh segment.

FOOD HABITS

From the gut contents (table I), it is apparent that the food of *Themisto* in the months of August and September consists mainly of the smaller crustacean plankton. It is probable that *Themisto* will eat any of the smaller forms, and that the frequency with which the various species are found in the stomach simply reflects the frequency with which they occur in the water. Numbers 8, 14 and 15

TABLE I. Gut contents of *Themisto libellula*.

No.	Date taken	Station	Length of specimen (mm.)	Gut contents
1*	14/8/40	Lake Harbour 4	32	*Calanus*, stages II and III. Remnants of 7 individuals recognizable.
2	3/8/39	" " 8	30.5	Nil.
3*	31/7/40	" " 4	31.5	Copepod appendages; ostracod shell.
4*	14/8/40	" " 4	25.5	Decapod larvae.
5*	30/7/40	" " 4	30	Copepod appendages; ostracod shell.
6*	23/8/40	" " 11	29.5	Copepods.
7*	2/8/39	" " 4	30	Crustacean remnants, part recognizable as copepod.
8*	3/8/39	" " 8	27.5	Gastric apparatus of *Themisto*; other crustacean appendages.
9	31/7/40	" " 4	13	Nauplius larvae.
10*	31/7/40	" " 4	12.5	Small crustacean appendages.
11*	14/8/40	" " 4	17.5	Copepod remnants.
12*	31/7/40	" " 4	22	Nauplius larva; ostracod shell; other crustacean remnants; algae.
13*	30/7/40	" " 4	27	Crustacean remnants.
14*	30/7/40	" " 4	25.5	Pharyngeal plates of *Themisto*; gregarines in anterior midgut.
15*	8/8/40	Frobisher Bay	17.5	Crustacean remnants, including 4th thoracic leg of *Themisto*.
16*	3/9/39	Arctic Bay	20.5	Unidentifiable detritus in foregut; small brown algae in hindgut. No sign of zooplankton.
17*	12/9/40	River Clyde	21	Unidentifiable detritus; no sign of zooplankton recognizable.
18	23/9/40	Pangnirtung	22	Crustacean remnants.
19*	23/9/40	"	20.5	Small copepods; other crustacean remnants.
20	23/9/40	"	21.5	Detritus including some algal cells. No zooplankton recognizable.

*Gregarine parasites in posterior midgut.

(table I) show that cannibalism occurs not infrequently, and numbers 12, 16 and 17 are evidence that vegetable matter also forms part of the diet, perhaps only at certain seasons.

Sixteen out of these twenty specimens contained numbers of gregarine parasites in the posterior midgut, many of them attached to the gut wall when dissected out. The size of the single trophozoite cell varied from about 0.1 mm.

to 0.6 mm., the largest measured being 0.63 mm. in length. Gregarines are commonly found in the Arthropoda, although the rate of incidence in the present material is very high. They appear to be restricted to the posterior midgut, those found in the anterior midgut of specimen number 14 (table I) being probably derived from another individual.

LOCAL DISTRIBUTION

Tables II and III are station lists for 1939 and 1940, showing all horizontal hauls made with the stramin net in Baffin island. They include data on the number of *Themisto* taken in each haul, depth, duration, time of day and light conditions. Depths are approximate only, being calculated from the length of rope and the angle of towing. *Themisto* was not found in any considerable numbers in the inner waters at Lake Harbour; stations 4, 8 and 10 were found to be the best stations for *Themisto* as for the majority of the plankton, and most of the collecting was done at these three stations.

Two facts are apparent from tables II and III: first that *Themisto* invades the uppermost layers in large numbers, and second that the population in the upper 30 to 40 metres becomes considerably reduced during the month of August. The one-metre ring-trawl is not a closing net, but with horizontal hauls of as much as 30 minutes' duration the proportion of the catch which enters the net on hauling up to the boat is very small, and by properly arranging the depths at which the tow-nettings are made much can be learned about the vertical distribution of the various species in the plankton.

Themisto libellula could be seen at Lake Harbour and Frobisher Bay right at the surface on many occasions, especially during bright sunshine; indeed there is evidence that *Themisto* is strongly positively phototropic, and that the stronger the light the closer *Themisto* is found to the surface in the daytime. It was regularly found to be closer to the surface than the rest of the zooplankton, with the exception of the Ostracoda. The copepods and the Hydromedusae, which dominated the plankton at different times, had a lower centre of vertical distribution than the hyperiids. On days of bright sunshine, the shallower hauls yielded larger numbers of *Themisto* than the deeper hauls. This is brought out in particular by the results obtained on August 5, 9 and 10, 1939, and on July 31 and August 8, 1940. On cloudy days *Themisto* sinks a little lower down in the water. It is supposed that the dark purple coloration is correlated with this heliotropism. Other planktonic forms commonly found at the surface on bright days are similarly protected by pigmentation (ostracods deep red, *Limacina* black).

The reduction of the *Themisto* population in the upper 40 metres of the fjord waters during the month of August was recorded in both years, and was confirmed (assuming that the species is common in July at other stations as at Lake Harbour) by the scarcity of the species at other points on the Baffin island coast later in the season, in September. Before definite conclusions as to the fate of this population can be drawn, further field work is required, including tow-netting in Hudson strait and off southeast Baffin island, and deeper hauling in the fjord waters themselves. It was not feasible to do any considerable amount of hauling at depths greater than 40 metres with the labour available and without a motor-

TABLE II. Ring-trawl station list, Baffin island, 1939. Light conditions: 1—bright sunshine; 2—intermittent sunny and cloudy; 3—cloudy, bright; 4—cloudy, dull.

No.	Date	Station	Depth (m.)	Duration of haul (min.)	Time of day	Light conditions	Number of *Themisto*
11	31/7	Lake Harbour 3	25	25	11.30	3	17
13	1/8	" " 4	25	30	11.05	1	321
14	1/8	" " 4	25	40	12.00	1	50
15	1/8	" " 4	25	30	13.55	1	114
16	1/8	" " 5	25	30	15.35	1	47
17	2/8	" " 7	25	30	10.00	3	3
18	2/8	" " 4	25	30	11.30	3	2181
21	2/8	" " 4	25	30	13.35	1	153
22	3/8	" " 4	25–30	30	11.10	1	256
23	3/8	" " 4	35	30	12.10	1	20
24	3/8	" " 8	30	30	15.00	1	0
25	3/8	" " 8	5	30	16.00	1	143
26	3/8	" " 8	0–1	30	16.45	1	209
27	5/8	" " 9	3–5	30	10.00		2
28	5/8	" " 8	15	30	12.30		32
29	5/8	" " 10	25	40	14.10		12
31	5/8	" " 10	25	35	15.25		10
32	5/8	" " 10	10–15	30	16.20		83
34	9/8	" " 4	20	30	11.30	1	20
35	9/8	" " 4	2–3	30	12.15	1	72
36	9/8	" " 4	10–15	30	13.45	1	67
37	9/8	" " 4	30	30	14.35	1	26
38	9/8	" " 4	15	30	15.25	1	14
38A	10/8	" " 4	20–25	30	11.05	2	3
39	10/8	" " 4	12–15	30	11.45	2	22
40	10/8	" " 4	3–5	30	12.30	1	148
40A	10/8	" " 4	1–2	30	14.15	1	2
41	10/8	" " 4	2–4	30	15.00	1	34
41A	12/8	" " 4	4–5	35	10.20	2	20
42	12/8	" " 10	20	30	12.00	2	10
43	12/8	" " 10	12–15	30	13.00	2	23
43A	12/8	" " 10	5–8	30	14.20	2	2
43B	12/8	" " 5	25	30	15.35	2	0
45	6/9	Pond Inlet	15	30	10.20	4	5
45A	6/9	Pond Inlet	8	30	11.10	4	1
49	3/9	Arctic Bay	15–20	30	20.00		13
51	3/9	Arctic Bay	10	30	21.10		8
57	13/9	Pangnirtung	15	40	11.35	1	16

TABLE III. Ring-trawl station list, Baffin island, 1940. Light condition scale as in table II.

No.	Date	Station	Depth (m.)	Duration of haul (min.)	Time of day	Light conditions	Number of *Themisto*
2	30/7	Lake Harbour 4	27	30	12.30	3	207
3	30/7	" " 8	11	30	14.30	1	86
5	30/7	" " 4	18	30	16.00	3	234
6	30/7	" " 4	28–30	30	16.55	3	58
7	31/7	" " 4	70–0†	10	12.30	1	11
8	31/7	" " 4	11	30	12.50	1	261
10	31/7	" " 4	6	30	14.15	1	370
11	31/7	" " 4	3–4	30	15.05	1	401
18	8/8	Frobisher Bay	26–32	30	10.20	1	24
20	8/8	" "	17–20	30	11.15	1	247
21	8/8	" "	8–10	30	12.10	1	143
22	10/8	" "	20–50**	15	22.00	dark	103
23	14/8	Lake Harbour 4	18	30	10.55	1	347
25	14/8	" " 4	30	30	11.40	1	114
27	21/8	" " 4	40	30	10.10	3	4
28	21/8	" " 4	30	30	10.55	3	17
29	21/8	" " 4	16	30	11.35	3	12
29A	21/8	" " 4	7	30	12.15	3	10
30	21/8	" " 10	18	30	15.00		2
31	23/8	" " 4	18	30	10.25	1	23
32	23/8	" " 11	30	30	13.30	1	13
33	23/8	" " 11	14	30	14.15	1	5
34	7/9	River Clyde	25	30	09.20		17
35	7/9	" "	11–14	30	10.05		2
38	11/9	" "	27	30	11.10		0
39	12/9	" "	20–23	35	13.10	4	7
40	12/9	" "	8–13	30	14.10	4	1
41	13/9	" "	32	30	09.00	3	0
45	23/9	Pangnirtung	16–20	30	11.10	3	0
47	23/9	"	35–40	30	11.55	3	2
47A	23/9	"	11–13	30	14.30	1	0
49	23/9	"	15–20	30	16.30	2	7
49A	23/9	"	20–25	30	17.30	4	2

†Oblique haul.
**Depth variable.

driven winch; and time and contingency did not allow the investigation of the open water of Hudson strait. It has already been mentioned that at 30 to 40 metres depth the plankton became scarce, but it is possible that there exists a separate deep-water plankton which was not touched by the present work. At all events, four main causes of the disappearance of the *Themisto* from the catches

might be suggested on general grounds: These hyperiids may (1) die a natural death, (2) be exhausted by predation by seals, (3) be carried out to open water, or (4) migrate actively, either horizontally or vertically.

The first possibility is rendered improbable by the fact that, as is shown below, the *Themisto* population had still some way to go before reaching breeding conditions. It is shown, furthermore, that two quite separate size-groups, two separate modes in the size-frequency curve, exist in these waters, and only the larger size-group were found in the stomachs of the ringed seal. Since both groups are involved in the reduction in population, it follows that predation by the seal cannot be postulated as a main cause. The third possible cause suggested above involves the agency of one or both of two currents—the outflowing surface current which is commonly found in fjords, caused by fresh water entering at the head of the fjord, a condition which exists at Lake Harbour, and the large-scale polar current which flows down the east coast of Baffin island and sends a branch into Hudson strait, flowing westward along the south coast of the island. If this latter current were involved, the expectation would be that the *Themisto* population would be replenished by the influx of individuals from further up-stream and from the open waters of Hudson strait, rather than the reverse. The extent to which such an open-water drift can affect the composition of the fjord waters over the annual cycle is not known, but in any case it is doubtful whether its activity could have any bearing on the particular problem of the movement of the *Themisto* population, since the current in question encloses the whole of the north, east and south coasts of Baffin island, and since *Themisto libellula* itself belongs to the water of the current.

The outflowing surface currents of fjords are normally very shallow, affecting only the top two or three metres. Knowing the habit of *Themisto*, however, in seeking surface water, especially on sunny days, this current offers a possible explanation of the reduction in population during August. An important point is that the rest of the zooplankton does not show the same decline in August, and the shallow surface current could act as a means of separating the *Themisto* population from the remainder. The surface current is without doubt active in removing the ice from the inlets, which is gone by the middle of July or earlier, and it is possible that the same current is responsible for the later removal of the *Themisto*. The ringed seal also leaves the fjords during July and August, to return later when the ice begins to form again.

Active migration by *Themisto*, vertical or horizontal, is not excluded by the present results, although downward migration by the whole population in summer, as opposed to downward migration by the adolescent group only, is considered improbable on general grounds. We are thus left with the possibilities that *Themisto* may be removed from the upper 40 metres either by vertical migration, or by the agency of the outflowing surface current aided perhaps by active horizontal migration. The emphasis is on the latter explanation.

THE BREEDING CYCLE

The size distribution of the *Themisto libellula* population at Lake Harbour in July and August is shown in figure 4. Altogether 3,188 specimens were measured, 1,877 from the 1940 catch and 1,311 from 1939, of which 110 consist of a

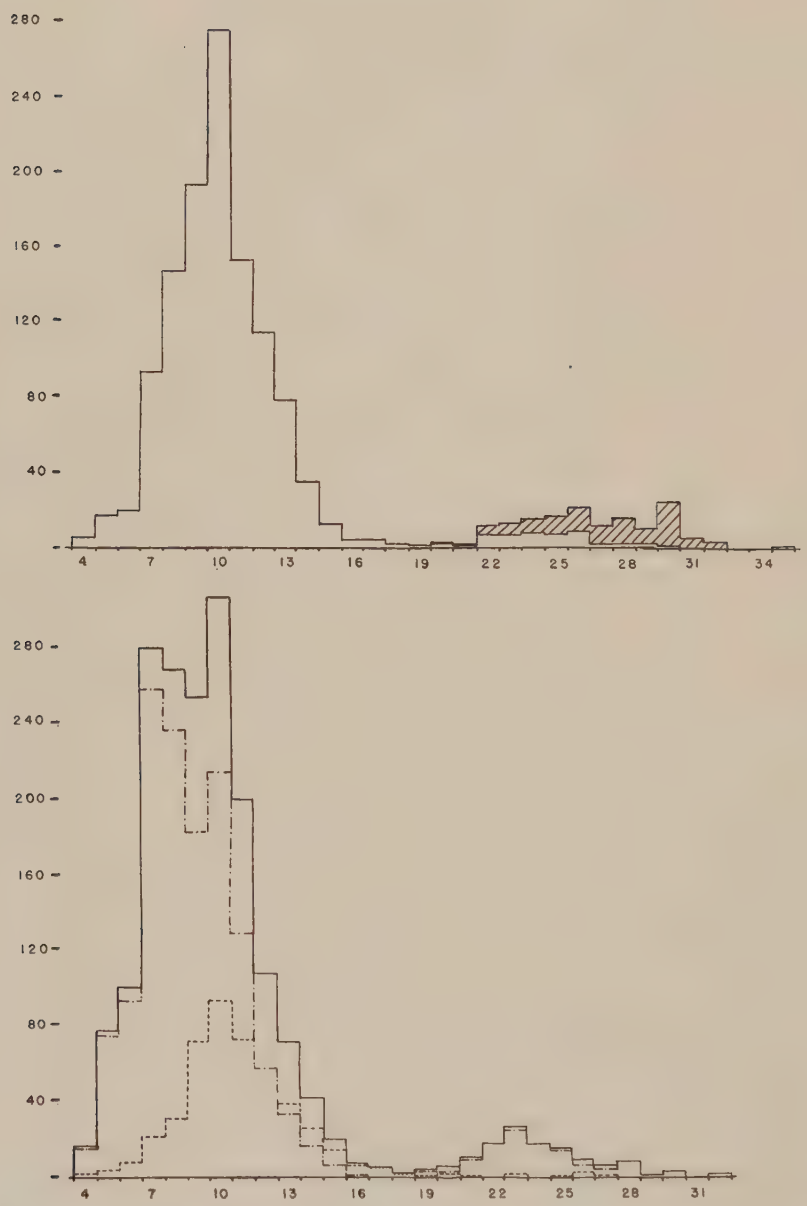

FIGURE 4. Size-frequency curves for *Themisto libellula*. Upper graph,—1939, Lake Harbour, taken between August 2 and August 12; shaded area—random sample from ringed seal stomachs, August 2-7. Lower graph,—1940, Lake Harbour; — - — - — taken on July 30 and 31; - - - - - taken between August 14 and August 23; ——— sum of both. Ordinates: number of individuals. Abscissae: millimetres length.

random sample from the contents of the ringed seal stomachs. A sample of 803 specimens was taken from haul number 18 (1939, table I), and the *Themisto* material from hauls numbers 31 to 43 (1939) was also measured. The whole of the 1940 Lake Harbour material was used. The measurements, from the front of the head to the tip of the uropods, were made to the nearest quarter-millimetre along a half-millimetre scale under a low-power binocular miscroscope. They were then referred to pivotal lengths centred on the integers and plotted by millimetre intervals (table IV).

TABLE IV. Size distribution in *Themisto libellula*, Lake Harbour.

Pivotal length (mm.)	1939			1940	
	Date: 2/8	5/8–12/8	2/8–7/8 (seal stomach contents)	30/7–31/7	14/8–23/8
4	3	2		14	1
5		17		74	3
6	10	10		93	7
7	57	36		258	21
8	107	40		237	31
9	136	57		183	71
10	216	59		214	93
11	111	42		128	72
12	74	40		57	50
13	47	31		33	38
14	26	9		16	25
15	10	3		6	14
16	2	2		1	6
17	2	2			5
18		2			2
19		1		3	1
20		2	1	3	2
21		1	1	9	1
22		7	5	17	
23		7	6	25	2
24		8	8	17	
25	1	6	10	14	1
26	1	8	13	6	3
27		2	10	4	2
28		1	15	8	
29		2	8	1	
30		1	24	2	1
31			5		
32			3	2	
33					
34					
35			1		
Totals	803	398	110	1425	452

The size frequency curves in figure 4 are quite obviously bimodal. In 1940, there was a gap of two weeks in the collecting at Lake Harbour, during which an expedition was made to Frobisher Bay. The *Themisto* caught before and after this interval are shown separately. It is apparent that, inside the left-hand mode of the whole curve (i.e. juveniles), the mode of the curve of the later juvenile group, taken between August 14 and 23, is somewhat to the right of the mode of the earlier group, taken on July 30 and 31. This difference is confirmed by statistical treatment, which shows that the means of the two groups are 10.679 and 8.610 respectively, difference 2.069, and that the standard error of the difference between the means is 0.1224 (Davenport and Ekas 1936, p. 37). The difference between the means is thus almost 17 times the standard error of the difference, which must certainly be considered significant. No such difference was found in the juvenile group of 1939, taken between August 2 and 12. In this case, however, the time interval was somewhat less than in 1940. There is evidence, therefore, that within three weeks in August the juvenile group can grow as much as 2 mm. A growth rate of this order does not of course disturb the bimodality of the curve as a whole.

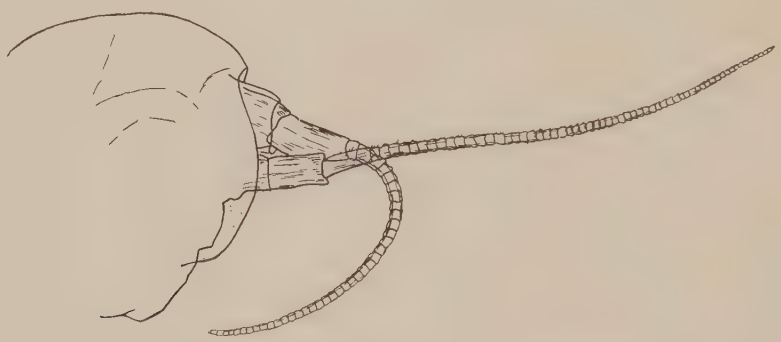

FIGURE 5. Antennae of adolescent male, body length 30.5 mm.

Bimodal size distributions of this kind are indicative of the presence of at least two separate age-groups in the population. It is shown below that the larger-size group is still sexually immature, and therefore it will be convenient to refer to the two groups as "juvenile" and "adolescent". It is conceivable that the adolescent group contains more than one age-group, but the breeding periods in *Themisto* in the arctic appear to be sufficiently far apart to make this improbable, and in any case the point does not disturb the main argument developed below.

That the "adolescent" group is in fact adolescent and sexually immature is demonstrated by an examination of the external sexual characters and of the gonads. The first antennae of the mature male reach to the hind margin of the first pleonal segment; the second antennae are longer still, reaching beyond the hind margin of the last pleonal segment. This was not the case with the largest males taken in Baffin island in 1939 and 1940. Figure 5 shows the antennae of one of the largest of these males, an individual measuring 30.5 mm. Though a little longer than the antennae of the female, they are less than a quarter of the

length of the mature male antennae. In the same way, the sexual papillae, or penes, of the male, carrying the openings of the sperm ducts on the 7th thoracic segment, were not visible in the males taken at Lake Harbour.

The brood lamellae, or oostegites, of the female show similar immaturity. The brood lamellae of the adult female are considerably longer than the gill-plates, as figured by Bovallius (1889). The lamellae in the Baffin island material first appear at a body length of about 25 mm.; those of the largest specimens taken have small and rudimentary lamellae at the base of the gill-plates, never exceeding one-third of the length of the latter.

The gonads begin to develop very early in the life of the arctic *Themisto*. Juveniles only 10 mm. long had gonads over 1 mm. in length, simple plates of small cells. An ovary in an individual 17.5 mm. in length measured 2.6 mm. In the largest females in the adolescent group, measuring from 28 to 31 mm., the ovary still consisted of a single layer of cells with large nuclei, similar to the juvenile ovary except for growth in size by lateral and longitudinal proliferation. The immaturity of the ovary, and its simple single-layer structure, was confirmed by sectioning. The ovary of a specimen 29 mm. long measured 6 mm. in length and 1.4 mm. in width, the cells averaging under 0.2 mm. in diameter.

The testes of the largest males showed some signs of development towards maturity, though mature testes were not found. The testis at this stage is elongated and flat, and translucent. It consists of a longitudinal cavity along the inner (mid-dorsal) side, a central zone of unidentified and undifferentiated cells which may form germ-cells later, and an outer edge of supporting tissue. Sections of the testes showed that the cavities were filled with small spermatocytes, and in one instance, in an individual 32 mm. long, a few spermatozoa could be seen in one region. The whole organ was 4 mm. long. The testis thus appeared to be in a more advanced stage than the ovary in female individuals of comparable length; but the complete absence of signs of maturity in the external characters of the males renders it impossible to regard the individuals in question as sexually mature. The testis has the appearance of being in process of very gradual development, and is in no way mature. Indeed, the development of the gonads is clearly very slow, even in comparison with the slow body growth, for they appear in the early life of the animal—in contrast to the condition in temperate waters, where the gonads of Crustacea usually appear quite late in life.

Bovallius (1889) gives the maximum length of the adult female as 60 mm. in the arctic, and according to Sars (1895) the length of the adult is 45 mm. Seal stomach material sent to the writer by Mr. John Cormack of River Clyde, however, shows that *Themisto* can breed in Baffin island waters at somewhat lesser body size. The material consists of the contents of a ringed seal stomach taken in March 1943 at River Clyde. The contents are exclusively *Themisto libellula*, and, besides an amount of broken individuals, include 10 mature females and 3 mature males, with externals almost intact. The internal organs are mainly digested. All the females have fully developed brood lamellae, agreeing with the figure of Bovallius, and three of them have developing embryos in the brood pouch, many of them in an advanced stage of development. The brood pouches of the other seven specimens are empty, and have either already shed their contents or have contained eggs which were digested by the seal. Eight specimens

range from 35 to 38 mm. in length; the other two measure 42 and 46 mm. The males measure 30, 32 and 36.5 mm.; all have the antennae and the sexual papillae fully developed. (They are thus mature at lengths little greater than the largest of the summer adolescent group at Lake Harbour). There are other heads of males in the material, with mature antennae. Besides these intact and broken mature specimens, there are a few smaller individuals, about 23 mm. in length.

The interpretation to be put on these facts becomes clear. There is a breeding period early in the year, beginning perhaps in March and April, very probably timed so that the young are freed about the time of the spring phytoplankton maximum. The presence of large numbers of quite small individuals in August suggests that this breeding period extends at least into June and perhaps into July. The absence of eggs from the microplankton in August and the immature condition of the larger-size group show that the breeding period does not extend into August. The juvenile group found at Lake Harbour in July and August are the offspring of this early spring breeding group, which presumably die after spawning. The smaller individuals in the March seal stomach contents, of size about 23 mm., represent the group which becomes the adolescent group of July and August. This assumes that the River Clyde conditions are comparable to those at Lake Harbour.

It remains to discover when the adolescent group was hatched, and when its own breeding period occurs; or, in other words, whether there is only one breeding period in the year, with a life cycle of two years, or two breeding periods and a one-year life cycle. The evidence available is inconclusive on this point, but it is possible, judging from the fact that mature males were found in March at body-lengths approaching the largest of the August adolescent group, and considering the condition of the testes in the latter, that sexual maturity follows soon after August, and that there is a breeding period in the fall. Relevant to this is Stephensen's (1923) recording of ten specimens of *Themisto libellula* about 40 mm. in length in a seal stomach in east Greenland, in latitude 66°N., on September 21, 1898. Without further material, however, it is impossible either to exclude or to establish the fact of an autumn breeding period.

The breeding cycle in *Themisto libellula*, therefore, in Baffin island waters, is of the alternating or two-phase type in which two age-groups exist at any one time, the older group not being parental to the younger. During the spawning period, three groups are present—the breeding group, the intermediate group and the newly-spawned brood. This type of breeding cycle has been found in other arctic planktonic organisms (Dunbar 1940, 1941b). It explains the mechanism by which the population is maintained at the high level which arctic conditions make possible, in spite of one important factor which would tend to act in the opposite direction, namely the slow growth caused by the low temperature. The alternating breeding cycle has the interesting corollary that the two broods are genetically separate, and failing some superimposed mechanism which might cause the over-riding of this separation (such as part of one breeding group holding over until the breeding period of the other group, or breeding twice) the genetic conditions are favourable for the development of differentiation between the broods which might in time lead to speciation. The environmental conditions of course remain the same for both broods.

In *Sagitta elegans* (Dunbar 1941) there is evidence of a gradient of breeding habit, ranging from the condition found in the English channel (Russell 1932) with several broods produced in succession in the year, through the type found in the gulf of Maine (Redfield and Beale 1940) and the gulf of St. Lawrence (Huntsman and Reid 1921) when there is an extended breeding season in the spring, summer and fall, to the arctic condition with the greater length of life and the alternating cycle. Material on the genus *Themisto* is less abundant, but the work of Bigelow (1926) suggests that the same is true of *Themisto* in the gulf of Maine. Bigelow finds that the breeding season in *Themisto compressa* and *T. bispinosa* extends over a large part of the year, the young being found in every month from February until October; there is evidence that the season continues further into the late autumn. Such a cycle, with an assumed maximum individual length of life of one year, would be expected to give size-frequency curves without marked modality, or none at all, and with left-handed, or positive, symmetry, in which the mean is greater than the mode.

SUMMARY

1. *Themisto libellula* material from Baffin island coastal waters is examined for the gross internal anatomy, the food habits, the local ecology in the summer, and the breeding cycle.

2. In the foregut, the chitinous and transparent proventriculus is found to extend backwards into the cavity of the midgut, forming a gastric apparatus consisting of two plates or sheets, a tapering and divided dorso-lateral plate and a shorter ventral plate. The significance of this apparatus, which resembles in principle the peritrophic membrane in insects and the Onychophora, is briefly discussed.

3. The food of *Themisto* in August and September is shown to consist in the main of the smaller crustacean zooplankton; plant matter was also found in the gut contents. There is a high incidence in the midgut of infection by a gregarine parasite.

4. *Themisto* has a higher centre of vertical distribution in the daytime than the bulk of the zooplankton and is normally found right at the surface on sunny days. There is a marked reduction in the numbers of the species during August, which is thought to be due not to natural death nor to predation by seals, but to the agency of the outflowing surface current normally found in fjords during the summer, and perhaps also to active migration. The possibility of vertical migration to deeper water is not excluded by the evidence available.

5. The breeding cycle in *Themisto* in Baffin island is of the alternating or two-phase type in which two generations exist simultaneously, except during the breeding period when there are three generations present. Failing some mechanism which might put the cycle out of phase in some way, the two generations are genetically separate. There is a breeding period in the early part of the year, extending perhaps from March to June, and there is a possibility of there being another breeding period in the autumn.

6. There is evidence that a gradient in the breeding cycle in the genus *Themisto* exists between the waters of the gulf of Maine and arctic waters, similar to that found in the Chaetognath *Sagitta elegans*.

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Effect of Abnormal CO_2 Tension on Development of Herring Eggs

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ABSTRACT

Laboratory experiments were made to ascertain whether an observed mortality of herring eggs under natural conditions could have been due to respiratory difficulties caused by low CO_2 or high O_2 content of the water. In water saturated with O_2, developing embryos tolerated a wider range of pH and CO_2 values than is prevalent in the sea. Hence the spawning ground mortality is presumed not to be due to such factors. Low pH retarded development of embryos and prevented successful hatching.

A high mortality of herring eggs took place on herring spawning grounds along the southeast coast of Vancouver island during the spring of 1942. Based on observations that excessive mortality was confined to eggs deposited below the low tide level, that at the time of the mortality there was a sudden rise in pH, and that there were records of similar mortalities under similar conditions in other places and years, the suggestion was made "that the death of the eggs below the low tide mark may have occurred because of respiratory difficulties resulting from low carbon dioxide or high oxygen content of the water" (Tester 1942).

To test the applicability of this suggested cause of mortality a series of laboratory experiments was conducted under controlled conditions. These were designed to determine the effect of abnormally low and abnormally high carbon dioxide content on the development and viability of herring eggs under conditions of high oxygen content (saturation). The results of these experiments form the basis of the present report.

METHODS

Material

Two separate lots of eggs were used in two similar experiments.

The first lot was collected at Skuttle bay, near Sliammon, on the eastern shore of the strait of Georgia, March 2, 1944, spawning having commenced on the previous day. The eggs, attached to eelgrass, were taken from below the low tide level and were transported in a wooden pail. During the trip, which lasted about 40 hours, they were moistened occasionally with seawater. On arrival, the embryos were at stage 2 (see below).

The second lot was collected at Matilda inlet on the west coast of Vancouver island, March 27, 1944, where fish in a small bait pound had spawned on kelp during the previous night. The eggs were transported as before, the trip lasting about 36 hours. On arrival, the embryos were at stage 1.

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Apparatus

The apparatus (fig. 1) in which the eggs (still attached to the seaweed) were placed, consisted essentially of aquaria immersed in a water bath, supplied with running seawater (4.4. ml./sec.) and devices for maintaining high and low pH and high oxygen content. The number of drops of saturated $Ca(OH)_2$ added per minute was carefully counted; the CO_2 was unmeasured and the only check

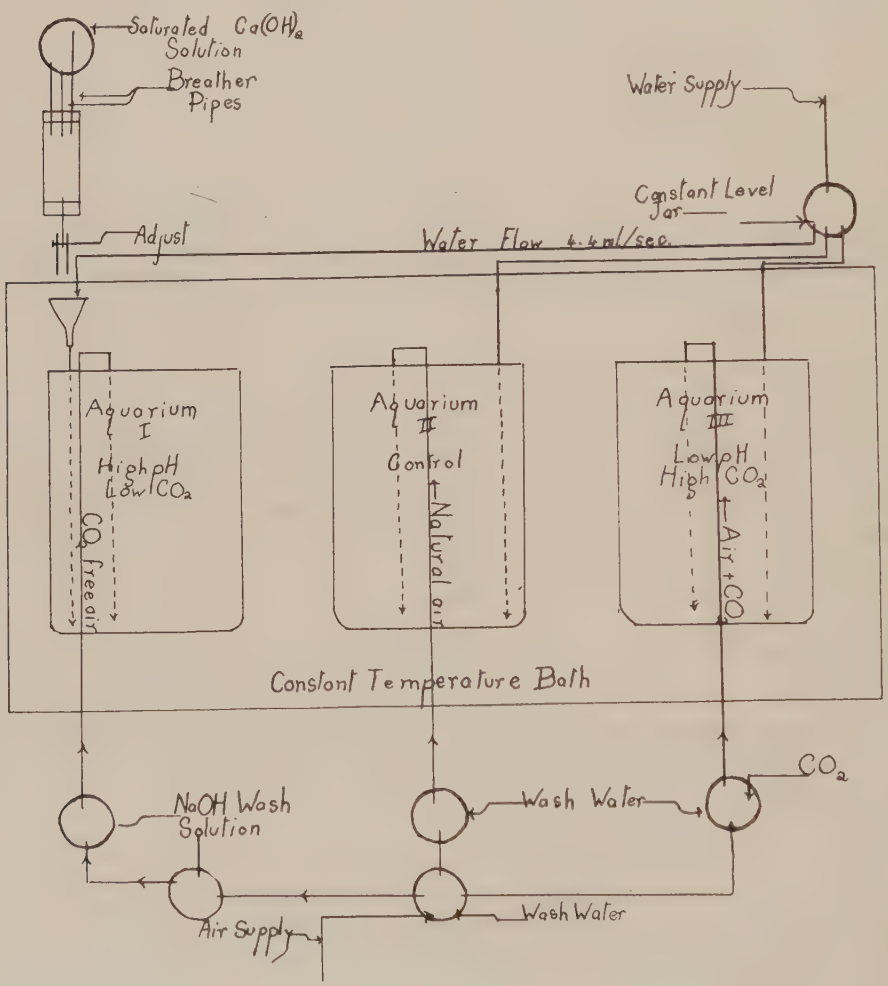

Figure 1. Diagram of apparatus.

on the amount added was the pH. It was intended as far as possible to keep the oxygen content up to saturation and to do this air was led from a compressor to the bottom of each aquarium and released as a fine spray of bubbles through the pores of an alundum crucible. In each aquarium the eggs, which tended to float, were kept under the surface with small stoppered bottles.

EXPERIMENT 1

Aqu. 1 — high pH; aeration, CO_2-free air; $Ca(OH)_2$ added. Aqu. 2 — normal pH; aeration, normal. Aqu. 3 — low pH; aeration, air with added CO_2 (unmeasured except for pH).

EXPERIMENT 2

Aqu. 4 — high pH; as in 1 above. Aqu. 5 — normal pH, as in above. Aqu. 6 — very high pH, otherwise as in 1 above.

OBSERVATIONS

Every morning a series of observations was made of pH, oxygen concentration, carbonate alkalinity and temperature. Additional temperature and pH readings were made during the daytime and evening in the low and high pH aquaria. The pH was determined electrometrically, the oxygen by the Winkler method, and the carbonate alkalinity by the method of Thompson and Bonnar (1931). Carbon dioxide, in parts per million (henceforth designated as p.p.m.) was calculated according to Thompson and Bonnar's formula relating pH and carbonate alkalinity.

Each day, a representative sample of eggs was taken, examined and preserved in a 10% solution of acetic acid before being re-examined at a later date. As stated by Hart and Tester (1934), this preservative helped to distinguish the living from dead eggs better than formalin. It probably would not distinguish the eggs that had died very recently and in which disintegration had not begun. Approximately 1% of the sample was made up of eggs listed as "doubtful" and a further 2% of eggs that, though living, showed no development. Development was indicated by the following arbitrary stages: I — one half of yolk covered by the cellular disc; II — embryo extending half way round the yolk; III — embryo extending almost completely around the yolk, head becoming well-defined, but tail still indistinct; IV — tail quite distinct and small portion of the tip free; eyes beginning to show; V — head and large part of tail free from yolk; body twisted so that tail passes over head; eyes heavily pigmented; yolk sac still relatively large; VI — embryo fully developed; yolk sac small and oval.

RESULTS

For the most part daily variation in pH, temperature, oxygen content and carbon dioxide content in each aquarium (table I) was within rather narrow limits and the ranges did not overlap. In experiment 1, aquarium 3 (low pH) there were wide fluctuations in pH and, in consequence, carbon dioxide content, over short periods of time (hours) because of erratic performance of a Kipp generator.

EXPERIMENT 1

In aquarium 1 (high pH) and aquarium 2 (control) development took place normally and at about the same rate, with eggs in the former completing their embryonic development one day earlier. This agrees with Loeb (1913), who showed that the addition of alkali favours the maturation process. The percentage of living eggs remained high (between 92 and 98%) in both aquaria

throughout the period until hatching started. Therefore no adverse effects were caused by a pH averaging 8.68 and rising as high as 8.96 with an attendant low carbon dioxide content of 0.132 p.p.m. However, in both aquaria only about 70% of the eggs hatched, the remainder gradually dying without hatching. Smith and Clowes (1924) note that "alkali-treated eggs are soft and more liquid than normal." This was found to be true. In addition to this a slimy substance coating the eggs may have inhibited the larvae from emerging. Possibly the presence of decaying vegetation contributed to this condition.

In aquarium 3 (low pH) it was noted by the fifth day that development was being retarded and this observation was verified by subsequent examination of preserved material. The percentage of living eggs throughout the period of

TABLE I. A summary of chemical data for each aquarium of experiments 1 and 2.

Aquarium	Av. pH (range)	Av. T. in °C. (range)	Av. O_2 in p.p.m. (range)	Av. CO_2 in p.p.m. (range)
		Experiment 1		
1	8.68 (7.93–8.96)	10.2 (9.5–10.8)	10.04 (9.82–10.18)	0.13 (uniform)
2	8.05 (7.94–8.11)	10.1 (9.5–10.8)	9.72 (9.36–9.90)	0.66 (0.572–0.881)
3	6.68 (5.12–7.88)	10.2 (9.5–11.0)	7.15 (5.05–9.30)	27.0 (7.52–67.76)
		Experiment 2		
4	8.67 (8.31–8.81)	12.4 (11.3–13.1)	9.78 (9.59–10.02)	10.13 (0.088–0.176)
5	8.12 (8.08–8.18)	12.4 (11.3–13.1)	9.45 (8.93–9.91)	0.66 (0.62–0.70)
6	8.87 (8.58–9.12)	12.4 (11.3–13.1)	9.67 (9.20–9.93)	0.088 (0.000–0.264)

development was almost as high as in aquaria 1 and 2 (82 to 96%) showing that the low pH and attendant high carbon dioxide content did not produce an excessive mortality. However, at the time that the eggs in the other two aquaria were hatching no larvae were observed in aquarium 3 (a number, dead and deformed, were found after the experiment was completed and the aquarium emptied). The eggs were turgid (noted by Smith and Clowes 1924) and not coated with slime, in contrast to those in the other aquaria. Failure to hatch and eventual death were doubtless related to the retarded and abnormal development induced by either the acidity or by the high carbon dioxide content, or, quite possibly, by the low rate of oxygen consumption induced by the high carbon dioxide content. Root (1930) observed that "the rate of oxygen consumption

of fertilized *Arbacia* eggs is depressed by carbon dioxide at all of the tensions studied." Burfield (1928) noted that small amounts of carbon dioxide profoundly decreased the rate of oxygen consumption of plaice eggs. Clowes and Smith (1922) found that the development of echinoderm eggs was inhibited by carbon dioxide at pH values which normally are without effect upon cleavage, and Whitley (1905) found that a disturbance toward the acid side is much more fatal than the opposite. In the present experiment the embryos were shortened and thickened, much more opaque in appearance than normal embryos, and had a dwarfed appearance. The yolk was relatively large and barely encircled by the embryo.

Experiment 2

This was undertaken to check on the results from aquarium 1 (high pH) and to determine any effects from even higher pH conditions.

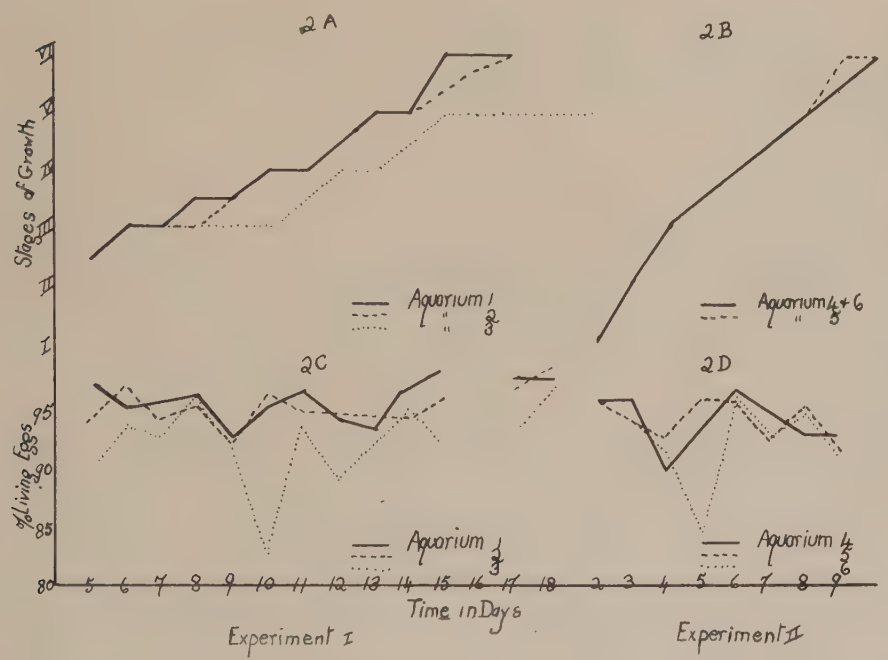

Figure 2. 2A and 2B—Development of herring eggs in the first and second experiments. 2C and 2D—Result of random sampling of herring eggs to determine the percentage alive (neither "dead," "undeveloped," nor "doubtful") until ready for hatching. Seeming inconsistencies are obviously sampling errors.

In general, apart from a more rapid development due to higher temperatures, the results for aquaria 4 (high pH) and 5 (control) were similar to each other and to those of aquaria 1 and 2 of the first experiment, with the same small mortality during embryonic development, with about 30% of the eggs failing to hatch, and with the eggs becoming flaccid and covered with a slimy coating.

In aquarium 6 (very high pH) development and mortality were similar to

those in aquaria 4 and 5 until the day before hatching. During the night the pH rose to 9.12 and only 40% of the eggs hatched. Usually the seaweed occupies the top third but in this aquarium part of it settled to the bottom and on this the mortality was as high as 100%. It was assumed therefore that, at a pH between 9.0 and 9.12, either a point was reached beyond which the eggs could not survive, or, that the kelp lying on the bottom of the aquarium had reduced the circulation so that the free hydroxide present at this pH was more concentrated in the lower part and killed the eggs by chemical action.

DISCUSSION AND CONCLUSIONS

From the foregoing results it may be concluded that the mortality noted by Tester (1942) was probably not due to respiratory difficulties induced by a low carbon dioxide content associated with a high pH of the seawater. The mortality referred to took place during embryonic development. In the experiments, the developing embryos showed a wide tolerance for pH values averaging from 6.68 to 8.68 and ranging from 5.12 to about 9.00 and for carbon dioxide values averaging from 0.088 to 27.06 and ranging from 0.000 to 67.76 p.p.m. Values encountered in the sea (7.8 to 8.0 in winter, and 8.2 to 8.5 in summer), even during the spring plankton bloom when there is an abrupt rise in pH, are well within this range.

It seems to be a generally accepted theory that some carbon dioxide is essential for normal respiration. Why the eggs did not die when exposed to the very low carbon dioxide concentrations (in one case 0.000 p.p.m.) opens up a whole field of conjecture. One possible explanation is that diffusion of carbon dioxide from the egg to the seawater is reduced by the small surface area presented by eggs which are massed together, as they normally are when deposited on seaweed.

Under laboratory conditions about 30% of the eggs failed to hatch and eventually died for some unknown reason.

Low pH retarded development of the embryo and prevented successful hatching.

ACKNOWLEDGEMENTS

The writer wishes to acknowledge her indebtedness to Dr. A. L. Tester, who suggested the problem, provided the eggs and assisted in other ways, and to Mr. John P. Tully for advice on chemical analysis.

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L'Iodate de Potassium et le Glucose Comme Agents Inhibiteurs de la Decomposition du Poisson

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RÉSUMÉ

L'iodate de potassium et le glucose employés individuellement retardent la décomposition des extraits de filet de morue salée et du jus musculaire de morue fraîche. Le mélange des deux paraît être plus efficace. Le glucose apparemment retarde la décomposition parce qu'il subit une fermentation acide, et l'iodate de potassium parce qu'il agit comme oxydant, ou indirectement comme antiseptique.

Dans un travail antérieur (Labarre et Fougère 1944) il a été constaté que l'influence de l'iodate de potassium sur la décomposition des extraits aqueux de morue salée était assez appréciable pour en faire l'objet d'une étude séparée. Le présent travail consiste à étudier l'effet de ce sel et du glucose sur des extraits aqueux de morue salée, à expliquer de quelle façon ils agissent comme agents inhibiteurs de la décomposition, et par une expérience appropriée, démontrer la possibilité de leur application dans la pratique.

Plusieurs produits chimiques ont été suggérés dans le passé comme moyen de retarder la décomposition du poisson; tels sont, les acides boriques, tartriques et citriques; les benzoates, le peroxyde d'hydrogène, les nitrites et les nitrates de potassium. Tarr et Sunderland (1940), Tarr et Bailey (1940) ont fait la revue de la littérature conçernant ce sujet. Il est inutile d'en faire ici une répétition.

Il a été démontré toutefois par Bailey et al. (1942) et Belfer et al. (1943) que l'iodate de potassium peut inhibiter l'activité de certains enzymes protéolytiques présents dans le foie de porc, le ver de terre, ainsi que dans les œufs et l'hépato-pancréas du homard. Sigurdsson et Wood (1942) ont étudié la fermentation du glucose par des suspensions bactériennes isolées de la chair de morue en voie de décomposition. Ils rapportèrent que le glucose subit une fermentation acide avec production d'acide lactique et formique, et que le pH du système sous observation diminuait considérablement durant la fermentation.

MODE D'EMPLOI

LES EXTRAITS

Tous les extraits utilisés au cours de ce travail ont été faits à partir de filets de morue salé du commerce. La morue salée a été choisie pour assurer des conditions plus faciles de travail.

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Les filets ont été d'abord broyés à travers un hache-viande. Cent grammes de tissus ainsi broyés ont été ajoutés à un litre d'eau distillée à température normale. Après avoir été agités pendant cinq minutes, ils ont été filtrés à travers un coton double.

En général, chaque extrait a été divisé en plusieurs portions dont chacune a été traitée suivant les essais envisagés. Ainsi, une expérience a consisté à suivre la décomposition à 40°C. d'un extrait divisé en quatre portions: la première contenait 0.1%, la deuxième 0.07% et la troisième 0.04% d'iodate de potassium. La quatrième servait de témoin.

Une deuxième expérience a consisté à suivre la décomposition d'un extrait ajouté de 0.5% de glucose à 25°C.

Une troisième expérience a été faite à la même température que la précédente, l'extrait étant divisé en quatre portions; la première contenait 0.1% de glucose, la deuxième 0.02% d'iodate de potassium et la troisième 0.05% de glucose et 0.01% d'iodate de potassium ajouté comme mélange. La quatrième servait de témoin. Le pH de chacune de ces portions a été observé avec l'espoir d'obtenir une idée du mécanisme de la conservation par le glucose et l'iodate de potassium.

L'action du mélange glucose-iodate a été étudié sur du jus musculaire de morue fraîche maintenu à 5°C. L'extraction du jus musculaire a été faite par la méthode employée par Beatty et Gibbons (1936).

La température de chacune des trois premières expériences a été choisie dans le but de créer des conditions telles, où les réactions de décomposition ne seraient ni trop lentes ni trop rapides. Chaque température, quoique son choix ne fut pas très rigoureux, a donc été choisie en tenant compte des concentrations des produits inhibiteurs employés et du sel que contiennent normalement les extraits de morue salée. La température de 5°C., dans le cas du jus musculaire de morue fraîche, a été choisie afin de représenter des conditions assez rapprochées de celles de l'entreposage des filets sur la glace.

La décomposition de ces extraits a éte suivie en déterminant chaque jour sur le liquide surnageant d'une aliquote centrifugée, la teneur en azote des bases volatiles ou, suivant le cas, en azote triméthylaminé.

PROCÉDÉS ANALYTIQUES

Bases Volatiles

Les bases volatiles ont été déterminées par une modification de la méthode de Parnas et Heller (1924) par entraînement à la vapeur à pression normale en présence de magnésie.

Cinq millilitres d'une aliquote centrifugée étaient introduits dans un appareil micro-Kjeldahl suivi de 10 millilitres d'une suspension aqueuse de magnésie puis distillés pendant 5 minutes, le distillat étant récupéré dans un millilitre d'acide sulfurique $\frac{N}{70}$. L'excès d'acide était à la suite titré par de la soude $\frac{N}{70}$ en employant comme indicateur, le réactif modifié de Taschiri, (Cole 1933 p. 327).

Triméthylamine

L'azote triméthylaminé a été déterminé par la mêthode de Beatty et Gibbons (1936).

Le pH

Les mesures de pH ont été faites au moyen de l'appareil à pH Coleman, modèle 3D à électrode de verre et muni d'un compensateur à température.

RÉSULTATS ET DISCUSSION

Les graphiques des figures 1 et 2 ont été tracés après deux séries d'expériences dont les résultats étaient en concordance. Les graphiques des figures 3, 4 et 5 ont été tracés après une seule série d'expérience.

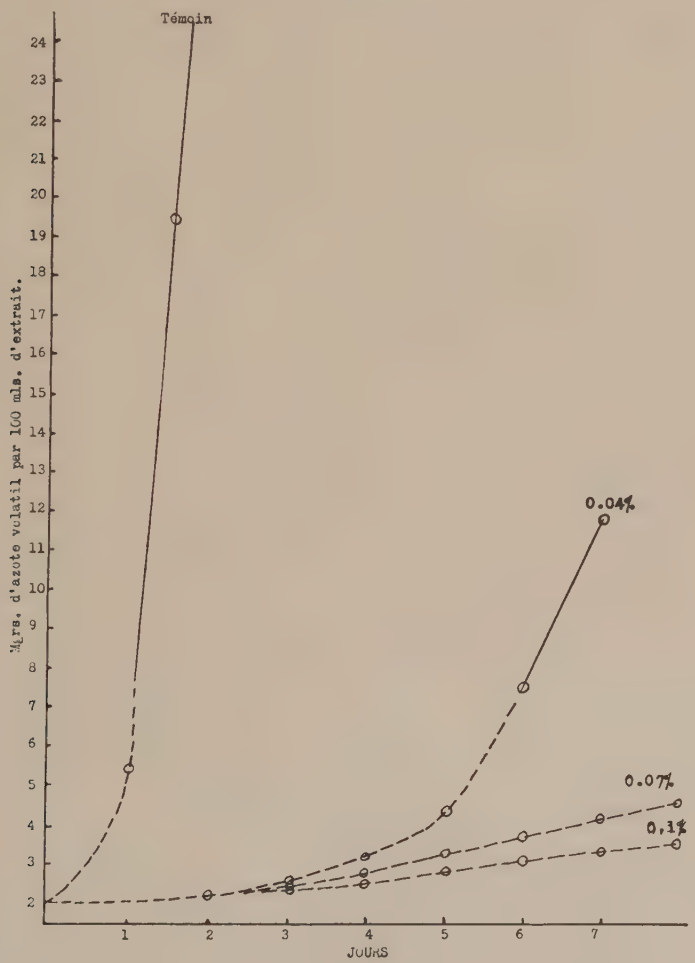

Figure 1. Influence de l'iodate de potassium sur la décomposition d'extraits aqueux de morue salée à 40°C.

Iodate de Potassium

La figure 1 fait voir l'influence de diverses concentrations d'iodate de potassium sur un extrait à 40°C. Pour le choix de cette température il a été tenu compte du fait que les concentrations d'iodate de potassium étaient assez fortes et que l'extrait soumis à l'expérience contenait du sel. Ainsi, à cette température

les réactions de décomposition pour les concentrations d'iodate utilisées et en présence du sel, sont ni trop lentes ni trop rapides, ce qui permet d'obtenir des résultats dans un temps relativement court. Cette expérience sert comme démonstration préliminaire de l'efficacité de l'iodate de potassium comme inhibiteur de la décomposition du poisson. La partie pointillée des courbes, comme d'ailleurs cela s'applique à toutes les expériences qui vont suivre, est située dans la zone où les extraits peuvent être considérés, au point de vue organoleptique, comme étant à l'état de fraîcheur. Il est alors évident qu'à la concentration de 0.04% d'iodate de potassium l'extrait s'est conservé à peu près six fois plus longtemps que le témoin et qu'à la concentration de 0.1% il s'est conservé au moins 8 fois plus longtemps.

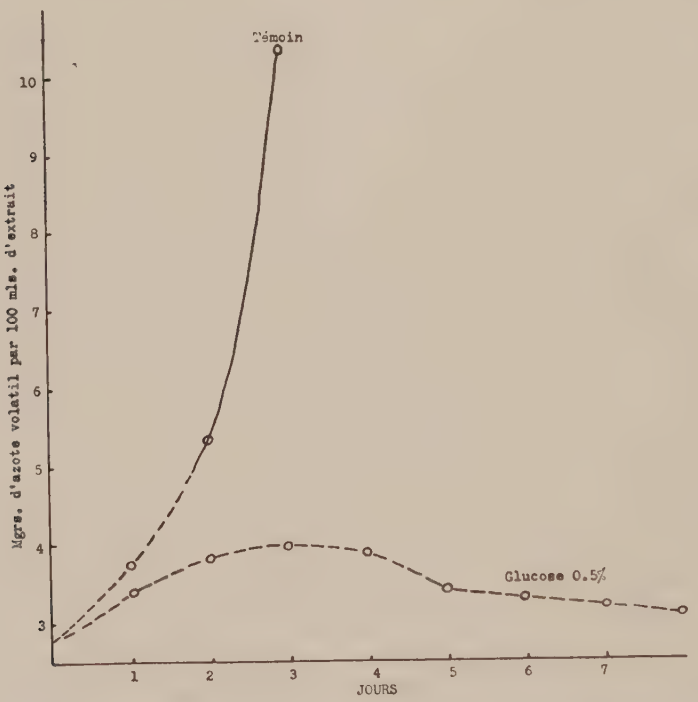

FIGURE 2. Influence du glucose sur la décomposition d'extraits aqueux de morue salée à 25°C.

GLUCOSE

Selon la figure 2, un extrait additionné de 0.5% de glucose et maintenu à la température de 25°C. peut se conserver pendant une période de temps assez appréciable. L'expérience que traduit la figure 2 ainsi que la suivante dans laquelle a été employé le mélange glucose-iodate, ont été faites à la température de 25°C. parce que, les concentrations des réactifs utilisés étant plutôt faibles c'est à cette température que les étapes des réactions pouvaient être suivis plus facilement et dans un lapse de temps ni trop long ni trop court. L'allure de la courbe, toutefois, présente un intérêt qui n'est pas du domaine de ce travail, mais il est évident que le glucose, quelles que soient les concentrations employées, donne une courbe d'allure toujours semblable.

Mélange Iodate-Glucose

Les résultats obtenus avec l'iodate de potassium et le glucose ont suggéré une étude sur l'influence du mélange de ces deux produits comme agent inhibiteur de la décomposition du poisson. Les résultats sont indiqués dans la figure 3. Le glucose et l'iodate de potassium, ajoutés en concentrations indiquées, possèdent chacun le même pouvoir inhibiteur, c'est-à-dire que chacun peut conserver l'extrait pour une période de trois jours; mais, lorsqu'ils sont ajoutés comme mélange, quoique leurs concentrations soient respectivement réduites de moitié, ils ont pour effet de doubler le temps de la conservation.

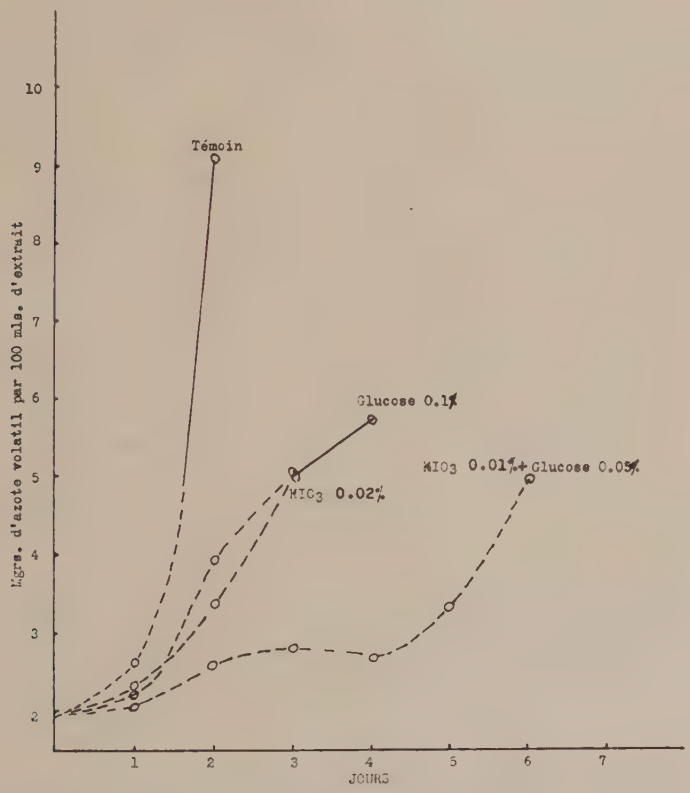

Figure 3. Influence de l'iodate de potassium et du glucose sur la décomposition d'extraits de morue salée à 25°C.

La variation du pH du témoin, figure 4, est la manifestation naturelle du vieillissement de la chair de morue (Nadeau 1939). Il est à remarquer que l'iodate de potassium change très peu la valeur du pH durant la première journée, valeur qui tend toutefois à se stabiliser pendant les deux journées suivantes. Cette stabilisation paraît être conditionnée par le pouvoir inhibiteur de l'iodate.

Nadeau (1939) a démontré que la chair de morue peut être conservée pendant de longues périodes de temps si le pH des tissus est maintenu à un niveau inférieur au pH 5. Or, dans le cas du glucose, le pH descend à 4.2 durant la première journée et monte immédiatement tout en restant inférieur au pH 5 jusqu'à la

quatrième journée. L'acide lactique et l'acide formique formés durant la fermentation du glucose sont tout probablement la cause du retardement de la décomposition.

Ajouté au glucose, l'iodate de potassium semble de nouveau agir comme stabilisateur du pH. Ainsi dans le mélange glucose-iodate, le pH demeure stable jusqu'à la quatrième journée. Durant cette période, de très petites quantités de bases ont été libérés au moins en quantités non suffisantes pour affecter le pH. En d'autres termes, la protéolyse et la réduction de l'oxyde de la triméthylamine ont été temporairement suspendus.

En outre, l'iodate de potassium est graduellement réduit pour libérer de l'iode, qui toutefois, n'est perceptible qu'en traces au moyen d'amidon, vers la fin de l'expérience, c'est-à-dire, vers la cinquième ou sixième journée. Il est possible alors que l'activité microbienne totale soit, dans certaines limites, retardés par l'iode naissant; mais, le rôle de l'iodate durant les quatre premières journées

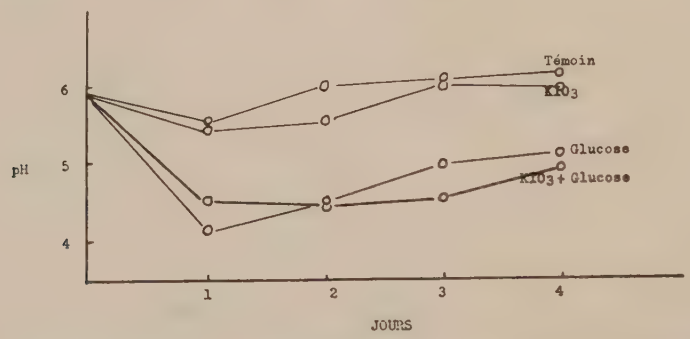

FIGURE 4. Influence de l'iodate de potassium et du glucose sur le pH d'extraits aqueux de morue salée à 25°C.

peut être analogue à celui de la quinone (Labarre et Fougère 1944) qui aussi est un inhibiteur de la décomposition et agit comme oxydant. Il est possible alors que l'iodate de potassium agisse aussi comme oxydant.

JUS MUSCULAIRE

INFLUENCE DU MÉLANGE IODATE-GLUCOSE

L'efficacité de ce mélange ajouté à du jus musculaire de morue fraîche à 5°C. est mise en évidence dans la figure 5. Cette température a été utilisée parce qu'il est à peu près celle d'entreposage des filets sur la glace. Les deux portions de l'échantillon additionnées de glucose et de l'iodate de potassium en concentrations respectives de 0.01% et 0.002% ne se conservent pas plus longtemps que le témoin, c'est-à-dire, environ 8 jours; mais, la portion à laquelle a été ajouté un mélange de ces deux composés dans les mêmes concentrations respectives se conserve à peu près deux fois plus longtemps, c'est-à-dire environ 18 jours.

CONCLUSION

En concentration de 0.4%, l'iodate de potassium peut conserver un extrait de filet de morue salée maintenu à 40°C. pendant six jours; en concentration de

0.1%, il peut conserver une portion du même extrait pour au moins 8 jours à la même température. Le témoin ne reste à l'état de fraîcheur que pour une journée.

Le glucose, ajouté en concentration de 0.5% peut conserver un extrait maintenu à 25°C. pour une période quatre fois plus longue que le témoin.

Le glucose et l'iodate de potassium ajoutée séparément en concentrations de 0.5% et 0.02% respectivement à deux portions d'un extrait maintenues à 25°C,. possèdent le même degré d'efficacité comme inhibiteurs de la décomposition. Avec le mélange des deux, en concentrations individuelles de moitié, le temps de la conservation est doublé.

Les variations du pH des extraits traités par le glucose, par l'iodate de potassium ou par le mélange glucose-iodate, incitent à une explication du mécan-

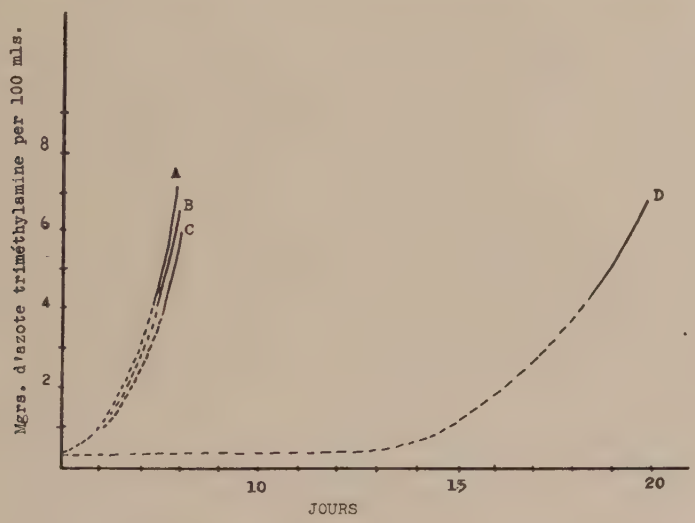

FIGURE 5. Influence de l'iodate de potassium et du glucose sur la décomposition du jus musculaire de morue à 5°C. A,—Temoin; B,—Iodate de potassium, 0.002%; C,—Glucose 0.01%; D,—B + C.

isme de la conservation. Le glucose, parce qu'il subit une fermentation acide, tend à maintenir le pH du système à un niveau inférieur au pH 5 où l'activité bactérienne est grandement retardé; l'iodate agit soit comme oxydant, ou comme antiseptique parce qu'il est lentement réduit pour libérer de l'iode naissant lequel est toxique pour les bactéries.

Dans le but de connaître les concentrations minima utilisables dans du jus musculaire de morue fraîche maintenu à 5°C., il a été constaté que 0.01% et 0.002% de glucose et d'iodate respectivement n'ont aucun effet sur la conservation. Le mélange des deux a toutefois comme effet de prolonger considérablement la conservation. En d'autres termes, les portions traitées individuellement par le glucose ou l'iodate ainsi que le témoin n'ont pû se conserver que pour une période de 8 jours, tandis que la portion qui contenait le mélange s'est conservée pendant 18 jours environ.

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Effect of Relaying and Transferring on Fatness of Oysters

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ABSTRACT

Down-river oysters are fuller-meated (fatter) than those up-river. When fishing and re-planting is done before fall fattening begins it affects fatness. Then, the disturbance of fishing and re-planting on the native bed improves fatness. Oysters transferred from up- to down-river beds show vast improvement, becoming fatter than native down-river stocks. Conversely, down-river oysters transferred up-river fail to fatten as well as the native up-river stock. Sometimes oysters fatten at water temperatures below 15°C., which was formerly considered the lower threshold.

As used here "relaying" means the re-planting of oysters (*Ostrea virginica*) on the bed from which they were fished and "transferring" means the re-planting of oysters on a bed other than that from which they were fished. Both relaying and transferring are common and necessary industrial operations, but there has been no general agreement either as to the season at which they can be best carried out or, in the case of transferring, as to the best choice of area for the re-planting. It is true that for most areas the quality of oysters both as regards shell characters and fatness of meats is low in up-river and high in down-river or bay areas. It is also known that oysters undergo annual cyclic changes in fatness (Medcof and Needler 1941). In 1941 and 1942 experiments were carried out at the Prince Edward Island Biological Station under the direction of Dr. A. W. H. Needler to see how fatness is influenced by relaying and transferring at different seasons.

PROCEDURE

The experiments were designed to show changes resulting from, (1) relayings on both up-river and down-river areas, (2) transfers both from up-river to down-river and from down-river to up-river areas, and (3) repetition of each of these experiments eight times at critical periods in the cycle of fatness changes.

In both 1941 and 1942 the Cooper bed in Bideford river was used as the down-river area. In 1941 the Ellis cove bed 2.6 miles (4.4 km.) above Cooper bed was chosen as the up-river area, and in 1942 the Sand bed 2.0 miles (3.4 km.) above the Cooper bed. The depth of water on all these at the places where the tests were carried out was five feet (1.5 m.) at mean low tide. The Cooper bed is of hard shell, the Ellis cover is of firm mud, and the Sand bed of a rather firmer sand-mud. In re-planting, each lot of oysters was merely thrown overboard from a dory into the water over the plot that had been marked off especially for it.

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The 1941 tests were preliminary in nature, although designed much the same as the full-scale experiments of 1942. The latter are described in table 1. As indicated, on May 8 a sample of "U" stock, adult up-river oysters, measuring $3\frac{1}{2}$ to 4" (9 to 11 cm.) in length, was fished from an undisturbed part of the Sand bed. All attached shells, small oysters, barnacles, etc., were removed, and all clustered oysters separated. These operations were carried out in the regular fashion practised by oyster farmers in working over their beds. Besides this a shallow groove was ground into the lower valve of each oyster near the hinge so that experimental animals could be identified at later dates. After being kept out of water for twenty-four hours without exposure to the sun, each sample was divided randomly into two equal lots. One lot, identified here as UD_1, was then transferred to a plot marked out for it alone on the down-river (Cooper) bed. The other lot, UU_1, was relaid on a similar plot on its native up-river bed. Simul-

TABLE I. Dates and numbers of transfers and relays of up-river and down-river oysters related to water temperature and phase in the annual cycle of fatnes changes.

Number of transfer or relay	Date 1942	Phase of fatness cycle on sampling date	Water temperature (°C.)	
			Actual	Trend
1	May 8	Middle of spring decline	below 15	rising
2	May 20	Early in spring rise	at 15	rising
3	June 9	Close to spring maximum	close to 20	rising
4	June 26	Middle of spawning decline	at 20	fluctuating
5	July 20	Post-spawning depression	above 20	fluctuating
6	Sept. 1	Middle of autumn rise	below 20	falling
7	Oct. 11	Autumn maximum	just below 15	falling
8	Nov. 9	After autumn decline and at level of winter plateau	below 5	falling

taneously a sample of "D" stock, Cooper bed oysters, was fished. One lot of this, DD_1, was relaid on the Cooper bed and the other, DU_1 was transferred up-river to the Sand bed.

On each of the dates listed in table I, succeeding May 8, this whole process was repeated, and the four new lots at each successive treatment were given an appropriate subscript number which appears in the left hand column of the table, to accompany their code letters. Thus by the end of the season there were thirty-two separate lots, but each lot could be completely described by two letters and a number. The first letter indicates the source of the oysters, the second the place where they were re-planted and the number, the position in the series of eight treatments. DU_6, for instance, was the sixth lot fished from the down-river and transferred on September 1 (table I) to the up-river bed. Again UU_4 was a lot fished from the up-river bed and relaid there on June 26.

On the date of each transfer and relay the fatness of the meats of each new lot and of each of all the lots handled up to that particular date was estimated by a determination of the average index of condition for ten oysters drawn at random from each lot, following the methods described earlier (Medcof and Needler 1941).

DISCUSSION OF RESULTS

Seasonal Change in Condition

In table II the first value of the index entered for each of the lots represents also the index, on that particular date, of the undisturbed native stock on the bed from which that lot was fished. Thus the values 114, 101, 107, 86, 90, 108, 117, and 126 taken from the DU and DD series of table II and the information in the dates column show how the index of Cooper bed oysters (D stock) changed with season. The course of change for the Sand bed oysters, U stock, can be similarly picked out from the values listed for the UU and UD series as 97, 112, 103, 82, 71, 95, 100, and 96. These two series show successive phases in the season's cycle of change in condition which have been described already for other areas (Medcof and Needler 1941). These phases are: (1) Spring decline (a brief

Table II. Index of condition of lots of relaid and transferred oysters on various dates in 1942 Up-river means the Sand bed and down-river, the Cooper bed.

	Up-river oysters transferred down-river.								Up-river oysters re-laid up-river							
Lot no.	UD_1	UD_2	UD_3	UD_4	UD_5	UD_6	UD_7	UD_8	UU_1	UU_2	UU_3	UU_4	UU_5	UU_6	UU_7	UU_8
Date																
May 8	97								97							
May 20	111	112							103	112						
June 9	129	121	103						109	113	103					
June 26	114	111	99	82					66	93	88	82				
July 20	98	97	92	87	71				95	87	87	82	71			
Sept. 1	131	158	128	136	117	95			110	113	133	118	113	95		
Oct. 11	135	142	135	141	141	118	100		130	115	115	121	101	105	100	
Nov . 9	115	136	121	131	130	119	117	96	120	125	118	118	117	113	110	96

	Down-river oysters transferred up-river								Down-river oysters relaid down-river							
Lot no.	DU_1	DU_2	DU_3	DU_4	DU_5	DU_6	DU_7	DU_8	DD_1	DD_2	DD_3	DD_4	DD_5	DD_6	DD_7	DD_8
Date																
May 8	114								114							
May 20	119	101							113	101						
June 9	111	106	107						107	119	107					
June 26	103	81	100	86					110	108	107	86				
July 20	93	86	83	96	90				97	95	107	97	90			
Sept. 1	122	102	127	120	113	108			111	126	126	133	121	108		
Oct. 11	119	103	115	120	119	121	117		126	127	127	125	133	113	117	
Nov. 9	113	110	112	117	121	123	119	126	118	105	117	124	123	122	133	126

period in May in this region); clear for the D stock whose index fell from 114 to 101, but too brief in the U stock to be detected in this experiment. (2) Spring rise to the spring maximum; slight in the D stock where the change was only from 101 to 107 but clear in the U stock where it was from 97 to 112. (3) Spawning decline; clear in both U and D stocks where the indices fell to 82 and 86 respectively. (4) Post-spawning depression, extending through the warm part of the summer when the index varied only slightly from the level reached during the spawning decline; clear in both stocks up to September 1. (5) Autumn fattening period. This was already underway on September 1, and culminated in the fall maximum recorded on October 11 when the indices for U and D stocks were 100 and 117. (6) Autumn decline from the maximum to the level of the winter plateau; this change, usually slight, shows up only in the U stock as a drop from

100 to 96. It is not at all apparent in the records for the D stock which, in fact, showed a rise in index.

These changes accord well with what might be expected from earlier records (Medcof and Needler 1941) except for the discrepancy just noted. This is emphasized because there were other cases in which there was a late autumn increase instead of a dropping off in the index as was expected from earlier findings. It was most conspicuous in lots that were transferred or relaid in late fall such as UU_7 and DD_7 whose indices rose from 100 to 110 and from 117 to 133 respectively, between October 11 and November 9. Available data indicate that late autumn fattening is unusual in undisturbed stocks.

Comparison of Undisturbed D and U Stocks

By comparing the index values on the same dates for undisturbed D and U stocks, it will be seen that, almost without exception, the D stock was the fatter. Its superiority was most apparent in late fall. On November 9, for instance, the indices were 126 and 96, respectively—a difference of 30 points. It was least apparent in late spring and summer. On June 26 the indices were 86 and 82—a difference of only 4 points.

The 1941 records show the same features. In November, 1941, the indices for undisturbed D and U stocks were 114 and 62 respectively—a difference of 52 points. In August the values were 104 and 78—a difference of only 26. Seasonal records of the condition of Bideford river oysters accumulated since the spring of 1939 up to the autumn of 1944 show that the difference in fatness between D and U stocks in 1942 was less than average.

It is known that spring water temperatures rise earlier and more quickly from 5 to 15°C. (and fluctuate between 15 and 20° for a longer period) in the upper than in the lower river and thus provide a shorter period of spring decline and a longer period of spring fattening for U than for D stock. Apparently then, the spring is the only season of the year when conditions for fattening are more favourable up-river. This explanation on the basis of temperature phenomena accounts for the late-spring and early-summer approximation of index values for U and D stocks even though the latter are higher in early spring.

The station's hydrographic records also show that the autumn water temperature falls below 20° earlier and fluctuates between 20 and 15° for a longer period in the lower than in the upper parts of the river. On the other hand, the lengths of the period when the temperature fluctuates between 15 and 5° are not conspicuously different in the two areas. Thus, although the periods of autumn decline in fatness are approximately equal, the period of autumn fattening is longer for D than for U stocks. This should partly explain the higher autumn values of the index for down-river oysters.

Effect of Relaying

If the indices for the undisturbed native stocks (table II) be compared with those for lots relaid earlier on the same beds, it will be seen that the former are almost invariably lower. For example, on July 20 the index of the D stock (Cooper bed) was 90 while that for lots DD_1, DD_2, DD_3 and DD_4 was 97, 95,

107 and 97 respectively. The average improvement resulting from relayings up to that date was 9 points in the index. The results for the Sand bed show the same trend On July 20 the index of the undisturbed U stock was 71 while that for lots UU_1, UU_2, UU_3, and UU_4 was 95, 87, 87, and 82, the average improvement from relaying being 17 points. From these and the other data it may be concluded that fishing, cleaning, separating and re-planting oysters on their native beds improves their chances for fattening and that the improvement is likely to be greater on up-river than on down-river beds.

The basis for these conclusions has not been subjected to experimental investigation, but available information suggests plausible explanations for them. It has been observed that after re-planting, oysters sit higher on the bottom than they did previous to being fished. Besides this, it is known that under ordinary conditions feeding and fattening of oysters improves with current speed and that current speed increases with distance above the bottom. From this we may conclude that in their raised position relaid oysters enjoy better internal and external water circulation than they did before being fished and that it is better circulation that permits the subsequent improvement in fatness which has been demonstrated.

It has been observed also that undisturbed oysters on relatively soft bottom like the Sand bed are deeper in the soil than are those on harder bottom like the Cooper bed. In spite of this they do not seem to settle back into the soil to any great extent during the first season after they are fished and re-planted. This circumstance suggests that the primary factor responsible for the buried condition of undisturbed U stocks is silting. Regardless of what the cause of burial may be, the point of importance here is that fishing and re-planting affects a greater change in level, relative to the bottom, for U than it does for D stocks, because Cooper bed oysters sit high on the bottom both before and after fishing and replanting. It is reasonable to assume that the improvement in water circulation and chances for fattening are directly proportional to the change in level on the bottom produced by handling. If this is true it would explain why Sand bed oysters benefited more from relaying than Cooper bed oysters, as was illustrated above using data for July 20.

The records show that the date of the relaying regulates the amount of the resulting improvement in fatness to some extent. The mean of the ten October and November values (table II) of the index for lots UU_1 to UU_5 which were all relaid before the fall fattening period began, was 118. The mean of the five values for the same months for lots UU_6 to UU_8, which were relaid after fall fattening began, was 105. In the DD series the corresponding means were 123 and 122 respectively. In the 1941 experiment the corresponding pairs of means for the UU and DD series were 71 and 65, and 121 and 113 respectively. These data clearly show that the maximum advantage provided by fishing and relaying was obtained when the handling was done before fall fattening began.

Effects of Transferring

A. transfers from up- to down-river beds

When the October and November indices for the UD and DD series are compared it will be seen that the ten values for lots UD_1 to UD_5 are almost

invariably higher than the ten for lots DD_1 to DD_5, the means being 133 and 123 respectively. From this it is concluded that if they are moved before fall fattening begins, U stock transferred down-river become even fatter than native D stock that has had the advantages of fishing and relaying. In contrast, the five values for lots UD_6 to UD_8 are lower than those for DD_6 to DD_8, the means being 110 and 122. This is attributed to the fact that the fall fattening period was practically over before these lots of U stock were transferred and consequently they had no opportunity to catch up with the native DD stock in in the short time left.

B. TRANSFERS FROM DOWN- TO UP-RIVER BEDS

When the ten October and November indices for lots DU_1 to DU_5 are compared with those for UU_1 to UU_5, they are found to be somewhat lower, the means for the two groups being 115 and 118. From this it is concluded that if D stock is transferred up-river before fall fattening begins it is not likely to fatten well, and by the end of the season is liable to be even thinner than the native UU stock. The five October and November indices for lots DU_6 to DU_8 are higher than those for UU_6 to UU_8, the means for the two groups being 121 and 105. This situation is explained by the fact that these lots of D stock had already become much fatter than the corresponding lots of U stock before they were moved from the Cooper bed and that since the fattening period was practically over, little subsequent change of any kind was to be expected, regardless of how the oysters were treated.

It appears that conditions for autumn fattening are better down-river than up, but so far there is nothing to indicate what factors, other than water temperatures, are involved. In a series of manuscript reports since 1933 to the Fisheries Research Board on the hydrography of the Malpeque bay area, Dr. Needler has shown that the salinities at the Sand bed and Cooper bed levels of Bideford river are almost identical throughout the greater part of the year, ranging from 24 to 29 and from 25 to 30 per mille, respectively. It would therefore seem unreasonable to attribute the differences in the behaviour of transferred oysters to changes in salinity, which Hopkins (1936) found to be important in regulating feeding activity.

The results of the transfer experiments show that oysters become adjusted to the particular conditions of their environment in such a way as to alter their reactions to fishing and re-planting. Thus up-river oysters which are adjusted to the adverse conditions on their native beds, when transferred down-river are able to take better advantage of good conditions obtaining there than the native down-river oysters relaid on those very beds. Again, down-river oysters, which are adjusted to life under favourable conditions, when transferred to up-river beds, are less able to cope with the unfavourable conditions found there than are the native stocks which have always experienced them.

The importance to the oyster culturist of this and of other conclusions reached in this discussion are clear to those acquainted with the industry. They have been discussed elsewhere from this particular point of view (Medcof 1944).

CONCLUSIONS

Down-river oysters are almost invariably fatter than those up-river.

When carried out before fall fattening begins, relaying and transferring have important effects on the condition of oysters. At such times the three following statements hold true.

Fishing and relaying favours fattening.

Up-river oysters transferred down-river show a vast improvement in condition and often become fatter than relaid native down-river oysters.

Down-river oysters transferred up-river do not fatten so well as native up-river stocks relaid on the same bed.

From the last two conclusions it appears that oysters become adjusted to the particular conditions of their environment in such a way as to affect their reactions to different types of re-planting.

There is some indication that autumn fattening may go on at temperatures below that formerly considered the lower threshold.

The conclusions from these experiments have an important bearing on industrial practice.

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